Behavioural investigation into whether L-DOPA, the current 'gold standard' pharmacotherapy for Parkinson's disease can be improved by optimising its treatment strategies

Tayarani-Binazir, Kayhan Ashley

Awarding institution:
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Behavioural investigation into whether L-DOPA, the current 'gold standard' pharmacotherapy for Parkinson's disease can be improved by optimising its treatment strategies

Kayhan Ashley Tayarani-Binazir

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This is to certify that I have carried out the studies embodied in this thesis under the supervision of Dr Sarah Salvage and Professor Peter Jenner.

Kayhan A. Tayarani-Binazir

Abstract

Long-term use of L-DOPA in Parkinson's disease results in motor complications and a progressive reduction in clinical efficacy. No new pharmacologic agents and treatment options have been able to deliver a more effective treatment than L-DOPA and therefore L-DOPA treatment strategies could offer improved clinical outcomes. This led to the hypothesis that **L-DOPA, the current 'gold standard' pharmacotherapy for Parkinson's disease can be improved by optimising its treatment strategies.** Using validated animal models of Parkinson's disease, behavioural studies were performed to test this hypothesis to evaluate if we could: (1) potentiate the clinical response of L-DOPA by maximising the efficiency of peripheral decarboxylase (DDC) inhibition, (2) enhance the clinical response of L-DOPA through prodrug delivery and (3) optimise L-DOPA's clinical therapeutic window through combination therapy with dopamine agonists.

Firstly, it was shown that the efficiency of DDC inhibition could improve the L-DOPA response particularly when L-alpha-methyl dopa (L-AMD) was utilised. Secondly, the novel L-DOPA prodrug PRX 1354, induced improvement in L-DOPA motor function in MPTP treated marmosets but did not have the same positive effect on dyskinesia expression. Lastly, in the MPTP-treated common marmosets, L-DOPA combined with the dopamine agonist pramipexole resulted in improved motor function and a reduction of dyskinesia.

In conclusion, manipulating L-DOPA treatment strategies can improve motor function while reducing dyskinesia expression. These results suggest that optimizing the treatment of Parkinson's disease by improving L-DOPA treatment strategies could reduce the impact of dyskinesia in Parkinson's disease patients.

Contents

Table of figures and tables

List of abbreviations

5-HT	5-hydroxytryptamine
6-OHDA	6-hydroxydopamine
AADC	Aromatic L-amino acid decarboxylase
ADME	Absorption, distribution, metabolism and excretion
AIMs	Abnormal involuntary movements scale
ANOVA	Analysis of variance
AR-JP	Autosomal recessive juvenile parkinsonism
AUC	Area under the curve
BBB	Blood brain barrier
CNS	Central nervous system
COMT	Catechol O-methyltransferase
DA	Dopamine
DBH	Dopamine β hydroxylase
DDC	Dopa decarboxylase
DDCI	Dopa decarboxylase inhibitor
DRN	Dorsal raphe nuclei
GDNF	Glial cell line derived neurotrophic factor
GI	Gastrointestinal
GPe	Globus pallidus pars externa
L-AMD	Aldomet
L-DOPA	L-3,4-dihydroxyphenylalanine
MAO	Monoamine oxidase
MAO-B	Monoamine oxidase-B
MFB	Medial forebrain bundle
MPP^+	1-methyl-4-phenyl-2,3-dihydropyridinium ion
MPTP	1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine
NADH	Nicotinamide adenine dinucleotide dehydrogenase
NMDA	*N*-methyl-*D*-aspartate
nNOS	neuronal nitric oxide synthase
NO	nitric oxide
Ns	not significant

PD	Parkinson's disease
PET	Positron emission tomography
PINK1	PETN-induced protein kinase 1
PK	Pharmacokinetic
ROS	reactive oxygen species
RT	room temperature
S.E.M	standard error of mean
SN	substantia nigra
SNpc	substantia nigra pars compacta
SOD	superoxide dismutase
STN	subthalamic nuclei
TH	tyrosine hydroxylase
UPS	ubiquitin-proteasome system

List of publications

Design, synthesis and biological evaluation of peptide derivatives of L-dopa as anti-parkinsonian agents.
Zhou T, Hider RC, Jenner P, Campbell B, Hobbs CJ, Rose S, Jairaj M, **Tayarani-Binazir KA**, Syme A.
Bioorg Med Chem Lett. 2013 Oct 1;23(19):5279-82
Cited: 5 times

Benserazide dosing regimen affects the response to L-3,4-dihydroxyphenylalanine in the 6-hydroxydopamine-lesioned rat.
Tayarani-Binazir KA, Jackson MJ, Strang I, Jairaj M, Rose S, Jenner P.
Behav Pharmacol. 2012 Apr;23(2):126-33

Design, synthesis and biological evaluation of L-dopa amide derivatives as potential prodrugs for the treatment of Parkinson's disease.
Zhou T, Hider RC, Jenner P, Campbell B, Hobbs CJ, Rose S, Jairaj M, **Tayarani-Binazir KA**, Syme A.
Eur J Med Chem. 2010 Sep;45(9):4035-42

The timing of administration, dose dependence and efficacy of dopa decarboxylase inhibitors on the reversal of motor disability produced by L-DOPA in the MPTP-treated common marmoset.
Tayarani-Binazir KA, Jackson MJ, Fisher R, Zoubiane G, Rose S, Jenner P.
Eur J Pharmacol. 2010 Jun 10;635(1-3):109-16.
Cited: 8 times

Pramipexole combined with levodopa improves motor function but reduces dyskinesia in MPTP-treated common marmosets.
Tayarani-Binazir KA, Jackson MJ, Rose S, Olanow CW, Jenner P.
Mov Disord. 2010 Feb 15;25(3):377-84.
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To my dear daughter Nur, you are the light that I strive for…

Chapter 1

General Introduction

1. General Introduction

1.1 Parkinson's disease background

Since the first description by James Parkinson in 1817 of the illness that now bears his name, Parkinson's disease has been widely investigated to determine its cause and treatment. James Parkinson described tasks that were:

"accomplished with considerable difficulty, the hand failing to answer with exactness the dictates of the will"

(ELLIOTT et al. 1954)

Parkinson's disease is an age-related neurodegenerative disorder, with the average age of onset being around 65 years (Obeso et al. 2000; Phillipson. 2013). The key clinical manifestations of the disease include bradykinesia or akinesia, rigidity, resting tremor and postural instability (Wooten, 2001; Willis. 2013). Other presenting symptoms, which are common amongst the older patients but may not necessarily be related to the disease, can include depression, cognitive changes and dementia. In recent years, research has focused on understanding the impact of these clinical presentations in order to improve the quality of life for the patients (Chaudhuri et al. 2011).

1.2 The pathology and biochemistry of Parkinson's disease

The clinical pathology of Parkinson's disease is the progressive and selective degeneration of the pigmented dopamine containing neurons of

the substantia nigra pars compacta (SNc) that project to the caudate-putamen. The loss of this pathway results in the motor symptoms of Parkinson's disease.

However, there is also degeneration of other systems including the mesolimbic system, the noradrenergic locus coeruleus, serotonergic raphe nuclei (Halliday et al. 1990), cholinergic basal nucleus of Meynert and cerebral cortex (Obeso et al. 2000d). The neuronal loss is accompanied by the presence of eosinophilic proteinaceous inclusion bodies called Lewy bodies (Phani et al. 2012), which appear to be present in dying neurones.

1.3 The aetiology and pathogenesis of Parkinson's disease

Although the cause of the disease still remains unknown, neuronal loss has been linked to mitochondrial dysfunction, oxidative stress, glial mediated inflammation, iron accumulation, dysfunction of the ubiquitin proteasome system (UPS) as well as genetic influences (McNaught et al. 2006). What is known is that age and genetic pre-disposition are key factors to the development of the disease.

The motor symptoms of Parkinson's disease arise when ~50% of dopaminergic neurones within the SNc have been lost (Obeso et al. 2000) and are evident by motor deficits. Early symptoms can be seen prior to the motor symptoms and include loss of smell, taste and sleep disturbances. These non-motor symptoms are linked to the degeneration in non-motor areas of the brain (Chaudhuri et al. 2011).

1.3.1 Environmental factors

The cause of Parkinson's disease remains unknown, but there is some suggestion that environmental factors can contribute to cell death. For example, in the 1970's a by-product of heroin synthesis used by drug addicts in the southern state of California (Davis et al. 1979) created patients with all the characteristics of Parkinson's disease (Fiskum et al. 1999; Irwin, 1986). The identified substance, which was believed to be responsible for the parkinsonian transformation, was MPTP (1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine). These patients were referred to as 'frozen addicts' as they displayed a rapid development of severe Parkinsonism. Shortly after it was confirmed that MPTP was only the precursor to the real molecule that caused the effects seen, namely MPP^+. This promoted interest that there could be environmental causes for Parkinson's disease, although no similar toxin or factor has been conclusively identified in Parkinson's disease patients (Olanow et al. 1999). However, research has indicated various environmental factors such as well water, pesticides and herbicides that may be associated with the increased risk of developing Parkinson's disease (Betarbet et al. 2002) .

1.3.2 Genetic factors

Important discoveries over the past 2 decades have made valuable correlations between Parkinson's disease patients and genetic mutations. Pathological data from autopsies genotyped for Parkinson's disease-related mutations in alpha-synuclein, Parkin, PINK1, DJ1, LRRK2, and

glucocerebrosidase have been the primary focus of these investigations. Mutations in the α-synuclein gene on chromosome 4 have been identified in unrelated families with autosomal dominant inheritance patterns of Parkinson's disease (Riess et al. 1998) . α-synuclein was at a similar time also discovered to be a major component of Lewy bodies (Spillantini et al. 1997). Mutations in the gene responsible for encoding ubiquitin carboxyl-terminal hydroxylase L1 have also been discovered in other patients exhibiting dominant inheritance. This genetic mutation leads to a reduction in enzyme activity, and could be a contributing factor to Parkinson's disease development/ progression in these patients (Leroy et al. 1998). Parkin gene mutations are associated with autosomal recessive juvenile parkinsonism (Kitada et al. 1998). Parkin functions as an E3 (ubiquitin-protein ligase) and mutations of the Parkin gene lead to a loss of enzymatic activity *in-vitro* (Shimura et al. 2000).

These mutations are collectively of interest as they are all part of the ubiquitin-proteasome protein clearance system. Through this system, mis-folded, mutant or damaged cellular proteins are ubiquitinated, targeting them for destruction via the 26/20S proteasome (McNaught et al. 2001) . Failure of the UPS system can lead to protein accumulation, inclusion bodies and degeneration of dopaminergic neurons and in recent years has also been linked to other disorders such as lysosomal storage disorders such as mucopolysaccharidoses (Reiger et al. 2013). Indeed, this has been replicated with systemic administration of proteasome inhibitors in rats (McNaught et al. 2004) and also in the MPTP-treated common marmoset

model (Duty et al. 2011; Fornai et al. 2005) .

PINK1 (PTEN-induced kinase 1) gene mutations have been shown to be responsible for another rare familial form of Parkinson's disease. Whilst the role of this enzyme is not well understood, it appears to be mitochondrially located and have a role in neuroprotection (Valente et al. 2004) . Also, mutations in the DJ1 (Bonifati et al. 2003) and LRRK2 (leucine-rich repeat kinase 2, encoding dardarin) (Paisan-Ruiz et al. 2004) genes have been linked to development of Parkinson's disease.

These studies – along with the likely future discoveries of other gene loci associated with Parkinson's disease, the variable symptomologies involved in patients with these mutations, and our more limited knowledge of sporadic Parkinson's disease – have led to the growing opinion that 'Parkinson's disease' is actually a syndrome confounded by many causes and pathologies, with the likelihood that individual cases have an aetiology that is either primarily genetic or primarily environmental, but not solely one or the other (Calne et al. 2004).

The past 20 years have yielded progress in our understanding of the genetic basis for Parkinson's disease. There has been considerable progress in finding risk loci. To date, approximately 16 such loci exist; notably, some of these overlap with the genes known to contain disease-causing mutations (Singleton et al. 2013). These methods will continue to enhance our understanding of genetics and allow us to transform this knowledge into therapeutics to treat Parkinson's disease.

1.4 Basal ganglia and motor function

The basal ganglia are made up from five large, interconnected subcortical nuclei. The primary role of the basal ganglia is to integrate and process the huge amount of input received from limbic and associative cortices as well as from sensorimotor areas. It is the abnormal function or changes within the basal ganglia that are considered to be the major cause of the motor features of Parkinson's disease (Jenner et al. 2011).

These sub-cortical nuclei span the striatum (the caudate and putamen), the globus pallidus (GP) (pars interna and pars externa), the substantia nigra (SN) (pars compacta and pars reticulata) and the subthalamic nucleus (STN) (Obeso et al, 2000; Crossman. 2000). The basal ganglia are responsible for the execution of movement and the associated rewards of it but are not considered to cause the initiation of movement although they may play a role in movement preparation (Paradiso et al. 2003), but the role of the basal ganglia as yet is not understood in its entirety (Hauber. 1998).

The idea of movement needing to be carried out implies it has a purpose or reward and the process of movement is instigated in the association cortex areas and via mossy fibers is translated and inputted into the lateral cerebellum into simple motor cortex commands, which descend from the motor cortex, down the motor tract to the muscles where movement is executed (McAuley. 2003). As with any movement, sensory feedback is required to calibrate the movement in order to accomplish the designated task in the most precise way possible. There are possibly several pathways

which control movement as some movements do not need to be processed, such as the “fight or flight” reaction whereas some movements/ senses can be cognitively concentrated on (i.e. throwing a ball at a target 5 meters away).

Movement related inputs to the basal ganglia are from the cerebral cortex and enter the striatum via the corticostriate pathway. Corticostriate axons terminate at the medium spiny neurones in the striatum. Medium spiny neurones use γ-aminobutyric acid (GABA) as their neurotransmitter and act as an inhibitory output for the striatum (Rouse et al. 2000).

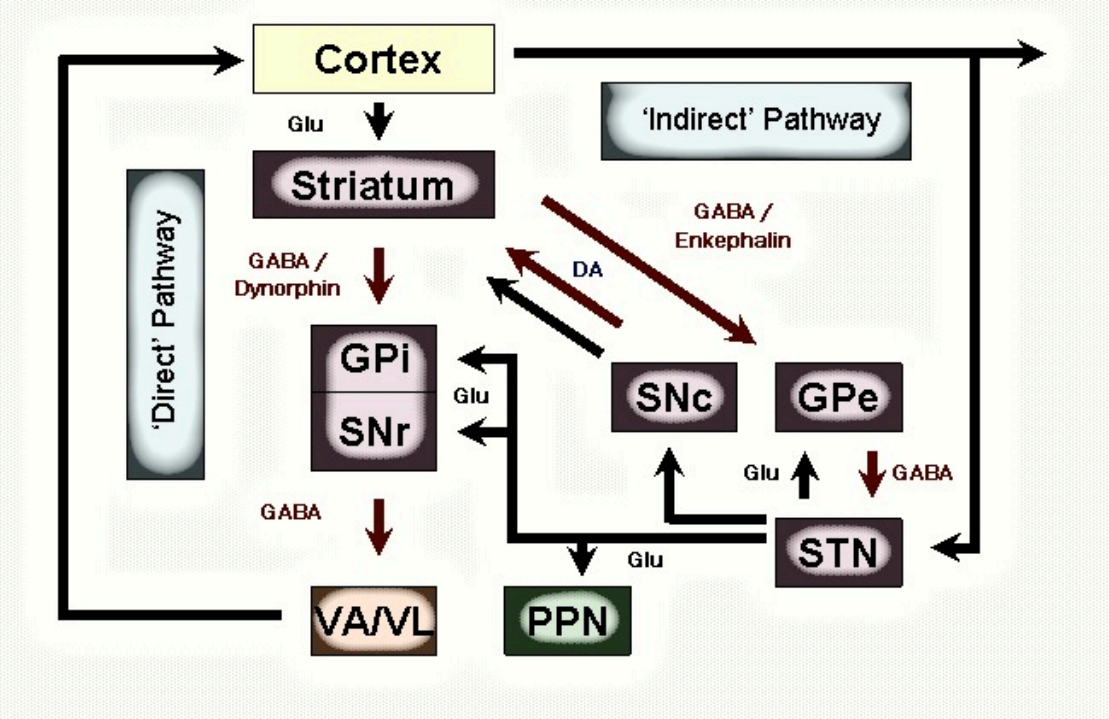
There are two types of medium spiny neurones:

1. Uses Substance P (SP) and dynorphin (DYN) as co-transmitters, express D1 DA receptors and project to the GPi and the SNpr.
2. Uses enkephalin (ENK) as a co-transmitter, expresses D2 DA receptors and projects to the GPe.

(Bonnet, Houeto. 1999)

The medium spiny neurones receive projections via the nigrostriatal pathway from the SNpc. The D1 expressed type neurone has its receptors positively coupled to adenylyl cyclase, the enzyme responsible for synthesising cyclic 5’adenosine monophosphate (cAMP) and enhances the excitatory cortical input effect whereas the D2 type neurone has its receptors negatively coupled to adenylyl cyclase and inhibits excitation (Gerfen. 2000).

The medium spiny neurones also have a third input from large aspiny interneurons which use acetylcholine (ACh) as an excitatory transmitter. AChesterase is compartmentalised in the striatum into the matrix and the striosomes. The matrix receives inputs from the cerebral cortex and sends outputs to the GP and the SNr. The striosomes receive excitatory inputs only from the pre-frontal cortex and project inhibitory signals to the SNpc. They seem to operate on a negative feedback loop onto the striosomes in the striatum as well as directly on the striatum itself (Gerfen. 2000) and from their give rise to the dopaminergic nigrostriatal tract (Albin et al. 1989).



(adapted from Lewis et al. 2003)

Figure 1 Basal ganglia circuitry in normal conditions

Motor cortex project in a somatotopic pattern to the posterolateral putamen, where they synapse through excitatory glutamatergic neurons onto the medium spiny striatal neurons. These striatal neurons are organised into two pathways: the 'direct' and the 'indirect' pathway. The direct pathway connects the striatum to the internal segment of the globus pallidus (GPi) and the substantia nigra pars reticulata (SNr). The GPi and SNr are the output nuclei of the basal ganglia (GPi/SNr) and project to the brainstem and the thalamus and from the latter to the cortex. The indirect pathway also connects the striatum to the output nuclei of the basal ganglia but these fibers first pass through synaptic connections in the external segment of the globus pallidus (GPe) and then the subthalamic nucleus (STN). Pedunculopontine nucleus (PPN) is involved in movement initiation and arousal.

The Direct Pathway

The medium spiny neurones using GABA/ SP/ DYN to inhibit GABAergic outflow of the GPi and SNpr to the thalamus make up the direct striatal efferent pathway. Cortico-striatal afferents control these neurons via glutamatergic stimulation resulting in an inhibitory effect on the GPi and because the GPi itself is having an inhibitory effect on the thalamus, it causes a disinhibitory effect upon the thalamus leading to excitatory inputs being sent from the ventral anterior/ ventral lateral nucleus of the thalamus to the SMA of the cortex which increases preparation for movement (Hauber. 1998). Delayed initiation of movement represents a dysfunction in the basal ganglia and is termed 'akinesia'.

Dopamine has an excitatory effect upon the GABAergic output neurons of the striatum that make up the start of the direct pathway. This excitatory effect is mediated predominantly though D1 receptors, and results in increased motor activity. Over activity of D1 can be associated with chorea like movements (Surmeier et al. 2007).

Indirect Pathway

The indirect pathway is controlled by corticostriatal glutamatergic input onto medium spiny efferent neurons which use the neurotransmitters GABA and ENK . These terminate at the GPe causing inhibitory signals to the subthalmic nucleus (STN). The STN then excites, via glutamate, the GPi and the SNpr, which have an inhibitory effect on the thalamus allowing it to filter unwanted movements. Therefore the indirect pathway is responsible

for reducing movements and is suppressed by inhibitory inputs from the SNpc (Obeso et al, 2000).

Dopamine has an inhibitory effect upon striatal Gabaergic output neurons associated with the indirect pathway. This inhibition is mediated through D2 receptors resulting in inhibition of the indirect pathway thus stimulating movement. This can often lead to more akinetic / dystonic movements (Surmeier et al. 2007).

Consequences of Dysfunction in the Basal Ganglia

Depleted DA levels due to the degeneration of the dopaminergic nigrostriatal tract are believed to be responsible for parkinsonian motor deficits. The loss of striatal dopaminergic innervation from the SNpc in Parkinson's disease results in reduced motor drive via increased activity of basal ganglia output nuclei. This is caused by reduced D1 mediated stimulation of the 'direct' pathway and reduced D2 mediated inhibition (disinhibition) of the 'indirect' pathway (Gerfen et al. 2000).

There is a strong body of evidence from animal models to support the idea that the STN and GPi are overactive in Parkinson's disease. In animal models including primates, measurements of 2-deoxyglucose uptake, used as a marker for synaptic afferent activity, support the model in terms of activities to the GPi and STN, as well as to the GPe, VA/VL thalamic nuclei and PPN (Mitchell et al., 1989).

Loss of dopaminergic neurons in the SNc reduces the normal inhibition of the nigrostriatal pathway on GABA-enkephalin neurons, which increases

their activity, thus over-inhibiting the GPi (Gerfen et al. 2000). The inhibitory tone of GPi on the STN is reduced and the STN increases its activity well over normal to excite the SNr.

Surgical lesions of the GPi (pallidotomy) and STN (subthalotomy) improve clinical symptoms in patients. High frequency deep-brain stimulation (DBS) of the GPi or STN also produces these effects, probably by inactivation of action potentials from these areas (Magarinos-Ascone et al., 2002). Electrophysiological studies in monkeys suggest the GPe may be receiving more excitatory input than normal (Levy et al., 1997). The increased 2-deoxyglucose uptake previously mentioned (Mitchell et al., 1989) could be partly caused by increased afferent activity from the STN or from modified activity at the postsynaptic element (Levy et al., 1997). It appears, therefore, that alteration of other inputs gives rise to STN hyperactivity. A nigrosubthalamic projection exists, and the STN appears to possess dopamine receptors, so loss of dopamine-mediated inhibition is a possibility (Smith and Kieval., 2000).

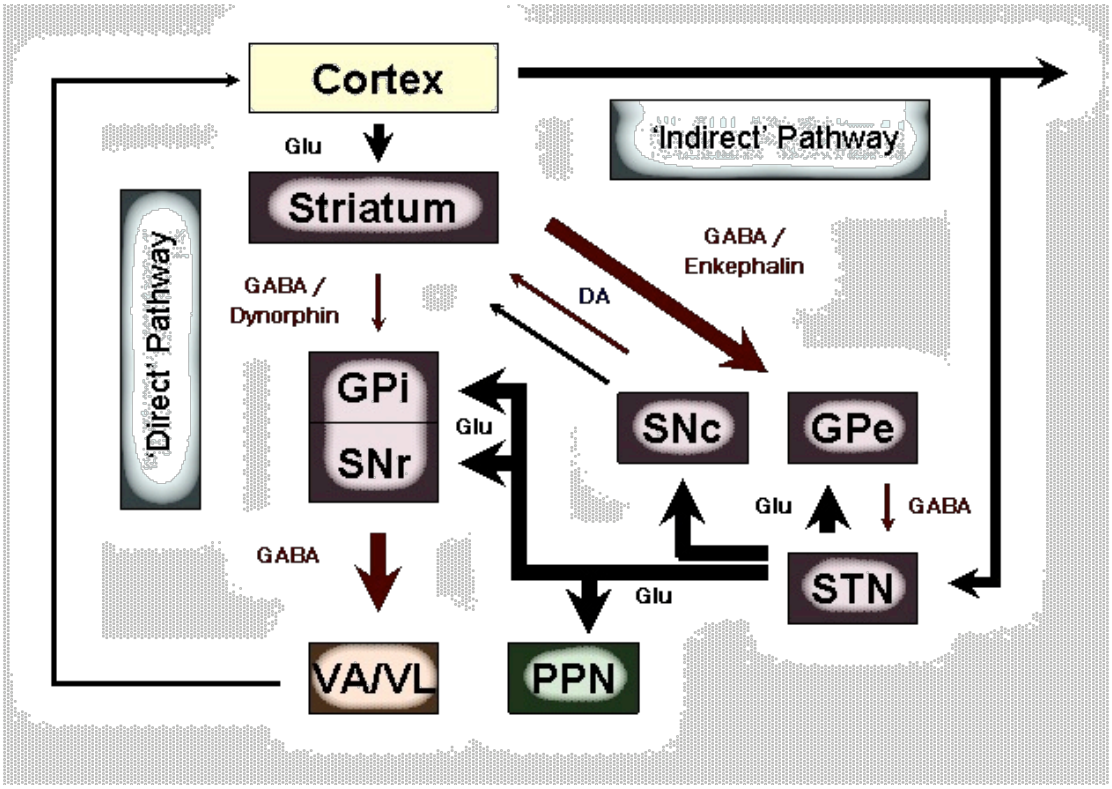
Cortical input to the STN is another possible route of excitation. Although it is agreed experimentally that STN and GPi/SNr output activities are generally increased in Parkinson's disease, these findings cannot account for the wide range of symptoms experienced by patients, and the variation between individual cases. This does also not account for the fact that surgical lesion of the STN or GPi improves Parkinson's disease symptoms without causing severe motor disturbances (Calne et al. 2004). It is increasingly becoming apparent that aberrant firing patterns, such as in

oscillatory activity, in the basal ganglia (rather than increased or decreased activity per se) are at the root of the parkinsonian state (Obeso et al., 2000).

Adenosine, an endogenous purine which is released from the nerve terminal to act as a neuromodulator as well as a neurotransmitter has also gained interest particularly the A2A antagonists, which are potentially interesting in PD treatment due to involvement with movement (Kulisevsky et al, 2002) largely because the receptors are preferentially located in the indirect pathway. In animal models of PD, it has been shown that adenosine A2A antagonists like istradefylline (Hauser et al, 2003) and theophylline (Kulisevsky et al, 2002) have increased movements in MPTP treated common marmosets. The adenosine A_{2A} receptor is abundant in the striatum where it modulates the output activity of gamma-aminobutyric acid (GABA) at the external globus pallidus (GP_e). Blockade of adenosine A_{2A} receptors increases movement whereas stimulation of the same receptor causes a reduction in movement.

A possible mechanism of A2A receptor antagonist action in Parkinson's disease has been proposed based on the involvement of striatal and pallidal presynaptic A2A receptors in the "dual" modulation of GABAergic synaptic transmission. Experiments with dopamine D2 receptor knockout mice showed that A2A receptors can function and anti-Parkinson's disease activities of A2A antagonists can occur independent of the dopaminergic system (Kase et al. 2003).

There are mixed feelings about the animal model results and clinical trials of adenosine A_{2A} antagonists in treating PD because although they have the ability to reduce 'off-time' there seems to be differences between the findings of various studies (Kanda et al., 1997, 1998; Kuwana et al., 1999). Most studies suggest that this class of drug shows no difference between placebo and drug effect (Kulisevsky et al, 2002). Indeed, A2A antagonists can often worsen tremor or akinesia and can cause nausea.



(adapted from Lewis et al. 2003)

Figure 1. 1 Basal ganglia circuitry in a parkinsonian state

In the Parkinsonian state, neurons in substantia nigra pars compacta (SNc) are lost. They cannot therefore excite certain neurons of the putamen and cannot inhibit others. This results in an excessive inhibition by putamen of globus pallidus externa (GPe), which can't inhibit the sub-thalamic nucleus (STN), which therefore activates the globus pallidus internus (GPi) and substantia nigra pars reticulata (SNr) too strongly. GPi and SNr inhibit the thalamus, which activates the cortex to start a movement. The thick arrows represent excessive stimulus and lighter arrows mean a deficient stimulus.

1.5 Treatments of Parkinson's disease

Parkinson's disease is one of the few neurodegenerative diseases in which the symptoms can be treated. The treatments are normally focused around dopamine replacement therapy although there are other treatment options.

1.5.1 Anti-muscarinic Drug Therapy

Before the introduction of L-DOPA in the 1960's (Brocks. 1999) the primary agents used to treat Parkinson's disease were anti-cholinergic drugs. Their use was justified based on knowledge that there was an imbalance between the dopaminergic inputs and the cholinergic interneurone activity within the striatum and reducing the cholinergic activity may help equilibrate this imbalance. In normal patients there is a tonic dopaminergic inhibitory tone of acetylcholine (Ach) release via D2 receptor stimulation whereas in Parkinson's disease patients where there is a loss of dopaminergic inhibitory input, hyperactivity of the cholinergic inter-neurones becomes apparent. The loss of this inhibitory input causes preferential signaling in the indirect pathway, which increases inhibitory inputs to the GPe. The GPe then becomes disinhibited leading to a reduced subthamamic nucleus (STN) output to the internal globus pallidus (GPi) and SNpr therefore causing a reduced drive (stimulation) from the thalamus to the motor cortex. The clinical manifestations of this reduction in the thalamo-cortical drive, is that of hypo-kinetic parkinsonian symptoms (Pisani et al. 2002).

It has been shown that anti-muscarinics have proven to be better on reducing tremor than other parkinsonian symptoms (Katzenschlager et al. 2003). The primary problem in using anti-cholinergics in Parkinson's disease is that they produce neuropsychiatric and cognitive disturbances, which are currently the main underlining reason why this drug therapy is often withdrawn in patients.

1.5.2 NDMA receptor antagonist - Amantadine

Currently, the only treatment intervention available for L-DOPA induced dyskinesia (LID) is the NMDA receptor antagonist amantadine (Shannon et al. 1987). Amantadine was first introduced to treat the symptoms of Parkinson's disease, but due to the high doses of drug required to have a therapeutic effect, it does result in significant side effects, which prevent many patient using it. Amantadine as an anti-viral drug has 4 properties, which make it a useful Parkinson's disease treatment. These are its weak NMDA antagonist properties, it increases dopamine release, it blocks dopamine uptake and it has anti-cholinergic effects. Following the discovery of an increased phosphorylation state of the NMDA glutamate receptors in striatal spiny neurons of dyskinetic MPTP-treated levodopa-primed monkeys, it was hypothesized that hyperactivity of glutamatergic transmission is a key mechanism underlying dyskinesia (Hallett et al. 2005). Amantadine's mechanism of action remains poorly understood, but it is one of the few noncompetitive NMDA antagonists that can be used in humans. It entered clinical trails during the late 1990s, which confirmed

its anti-dyskinetic effects in Parkinson's disease patients.

However, the beneficial effects of amantadine last less than eight months and subsequent withdrawal of amantadine therapy induces a worsening in dyskinesia (Thomas et al. 2004). In addition, amantadine has a number of adverse effects, including nausea, insomnia and various other peripheral and central disturbances, and it cannot be tolerated by all patients (Verhagen et al. 1998).

1.5.3 A2 Adrenoceptor antagonists

Non-dopaminergic treatment options are a key area of on-going research. One such area is of interest are the α2 adrenoceptor antagonists. Their empirical discovery showed that they have anti-dyskinetic actions when administered in combination with L-DOPA but not with DA agonists (Bezard et al. 2001). Noradrenaline (NA) is believed to compensate for the loss of DA actions in Parkinson's disease patients but the action of NA on dopaminergic neurones of the SNpc is not fully understood (Cathala et al. 2002). A2 adrenoceptors are considered to act as autoreceptors modulating the inhibition of NA release on pre-synaptic nerve terminals innervating the SNpc. NA causes the conductance of positive ions and increases the spontaneity of GABA release onto DA neurones.

This implicates NA as a regulator for the excitability of DA neurones.

Recent discoveries on the noradrenergic system in the brain have identified that a deficiency of NA originating from the Locus Coeruleus (LC) play a role in the progression of Parkinson's disease (Marien et al. 2004). The major implication of this is that apart from the obvious pathological deficiency in DA, other neurotransmitters may also prove to be beneficial therapeutic targets in Parkinson's disease treatment (Haapalinna et al. 2003).

1.5.4 Monoamine Oxidase (MAO) Inhibitors

With the discovery that MPTP is able to induce a parkinsonian state after its systemic administration, there has been a great interest in monoamine oxidase (MAO) inhibitors and in particular the MAO-B isoform which has been used even before the discovery of MPTP.

Dopamine is metabolised by MAO-B and COMT in the brain. Inhibition of these enzymes can potentiate the effect of dopamine formed endogenously or via L-DOPA. Both Rasagiline and Selegeline are known MAO-B inhibitors, which potentiate the effect of dopamine and produce a mild anti-parkinsonian effect (Rascol et al. 2012). When administered with L-DOPA, they can potentiate the adverse effects most probably linked to dopamine potentiation. In addition, Selegeline can be metabolised to amphetamine, which increases the release of dopamine (Dewey, Jr. 2004). There is some evidence that MAO-B inhibitors not only improve the symptomatic treatment of Parkinson's disease, but are also able to slow the disease process. However, clinical studies are equivocal, with no effect of

Selegeline and inconclusive results from the ADAGIO trial (assessing Rasagiline) (Rascol et al. 2012).

1.5.5 Adenosine A2A Antagonists

Adenosine, an endogenous purine which is released from the nerve terminal to act as a neuromodulator as well as a neurotransmitter has gained interest in Parkinson's disease treatment due to its involvement with movement (Kulisevsky et al. 2002). In animal models of Parkinson's disease, it has been shown that adenosine A2A antagonists like istradefylline (Hauser et al. 2003) and theophylline (Kulisevsky et al. 2002) have increased movements in MPTP treated common marmosets. The adenosine A2A receptor is abundant in the striatum where it modulates the output activity of the indirect gama-aminobutyric acid (GABA) output to the external globus pallidus (GPe). Blockade of adenosine A2A receptors increases movement whereas stimulation causes a reduction in movement (Morelli et al. 2009). Whilst the exact role of adenosine A2A antagonists is not clear, there is a growing body of evidence that if utilised correctly and early enough before LID, then they can reduce the associated dyskinetic effects (Kanda et al. 1998).

The findings from clinical studies in Parkinson's disease patients are equivocal. Adenosine A2A antagonists have been reported to reduce 'off-time' (Kanda et al. 1998a; Kuwana et al. 1999). However, most studies suggest that this class of drug shows no difference between placebo and drug effect (Kulisevsky et al. 2002). The concern with using this type of

drug therapy is that it can often worsen tremor or akinesia and can cause nausea (Richardson et al. 1997). Overall these pharmacological and genetic data provide evidence that striatal A2A receptors play an important role in the neuroplasticity underlying LIDs, supporting consideration of early adjunctive therapy with an A2A antagonist to reduce the risk of LID in Parkinson's disease.

1.5.6 Catechol-O-Methyl Transferase (COMT) Inhibitors

The use of L-DOPA and DA agonists restore motor function in Parkinson's disease patients/ animal models of Parkinson's disease to a certain level but the primary failing of L-DOPA in particular is the 'wearing off' effect (Stocchi. 2003). When Parkinson's disease patients are on L-DOPA during its clinically beneficial stages, they experience 'on-time'. This is when L-DOPA is active and has restored some of the motor control lost due to the denervation of the dopaminergic neurones. The 'wearing-off' stage is where the drug plasma levels begin to decrease giving rise to the reemergence of Parkinson's disease symptoms which is exacerbated by L-DOPA having a short half-life of 60-90 minutes (Koller. 2002).

After continued use, the L-DOPA response becomes gradually shorter due to the progress of the disease (McColl et al. 2002) so the 'wearing-off' stage becomes harder to estimate as to when it will begin but also the patient will experience a longer 'off-state' as well as the 'on-off' effect which can render a patient immobile at critical times. This often causes discomfort and can

lead to patients suffering from panic attacks when events (changes in mundane occurrences) arise (Dewey. 2004). It is for these reasons that pharmacological drug manipulation has begun to evolve for Parkinson's disease and one of the primary candidates are Catechol-O-Methyl Transferase (COMT) inhibitors.

Once in the systemic system, L-DOPA has two main routes of metabolism. These are through decarboxylation by dopa decarboxylase (DDC) or methylation by COMT. Due to the conventional use of carbidopa (standard form of DDCI) with L-DOPA the primary elimination of L-DOPA is carried out by COMT (Stocchi et al. 2003). In the brain and liver COMT degrades L-DOPA to its primary metabolite, 3-O-methyldopa (Galvez-Jimenez et al. 2000; Hanson et al. 2001). This metabolite does not undergo further metabolism and it is believed that due to its long half life (around 15 hours), it can accumulate in large quantities and its presence in blood levels can possibly explain the often poor clinical response to L-DOPA (Reches et al. 1982). This is because 3-O-methyldopa competes with L-DOPA for uptake into the central nervous system. The two main COMT inhibitors that are clinically used are entacapone (peripherally acting) and tolcapone (centrally acting) with the latter being the more potent drug, which can increase the half life of L-DOPA by approximately 40-80% (Gasser et al. 1999). Although these drugs have the ability to increase 'on-time', they only reduce 'off-time', they don't eliminate it.

1.5.7 L-3,4-dihydroxyphenylalanine (Levodopa or L-DOPA)

Despite being introduced 50 years ago L-DOPA the immediate pre-cursor of dopamine (DA) (Bartoszyk et al. 2003) is currently the 'gold standard' of drug treatment for Parkinson's disease.

L-DOPA crosses the blood-brain barrier via the large neutral amino acid transporter (LAT), and is taken into cells via a carrier similar to the LAT (Ahlskog 2003). In the brain it is enzymatically converted to dopamine by a dopa-decarboxylase and acts to replace the reduced levels of endogenous dopamine. However, L-DOPA, taken orally, is metabolized to dopamine in the periphery, allowing only a small amount (1%) of the total oral dose to penetrate the blood brain barrier (BBB). This not only reduces the efficacy of the treatment, but also induces unwanted side effects including nausea and vomiting due to the action of dopamine on the area postrema. To prevent the peripheral metabolism of L-DOPA, it is necessary to inhibit the action of the converting enzyme, dopa-decarboxylase. To this end, a dopa-decarboxylase inhibitor (DDCI), such as benserazide or carbidopa, is routinely given in combination with L-DOPA (Madopar® or Sinemet® respectively) (Da et al. 1987b). These inhibitors do not block all the peripheral metabolism of L-DOPA but ensure a significant amount reaches the target area in the brain without themselves readily crossing the blood brain barrier, allowing metabolism to DA.

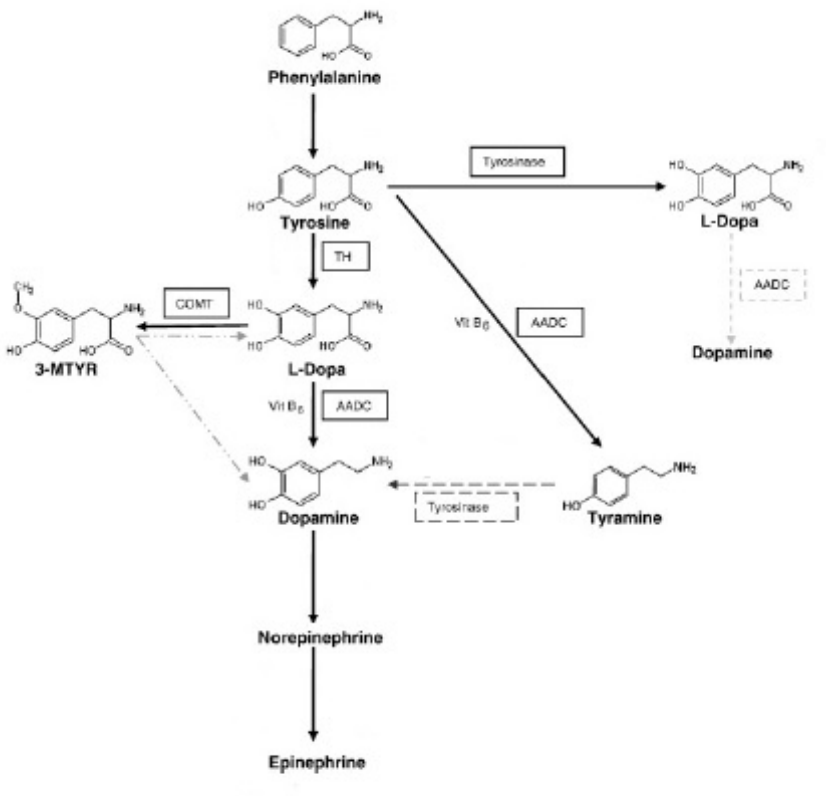


Figure 1.2 Metabolic pathway of L-DOPA by both DDC and COMT and its subsequent metabolites. The metabolism of L-DOPA via DDC to yield DA and via COMT to yield 3-O-Methyl-DOPA (adapted from Ngwuluka et al. 2010 and from Sozio et al. 2012).

During the late 1980s, ‘‘controlled-release’’ (CR) oral formulations of levodopa (Sinemet CR and Madopar HBS) were developed in another attempt to increase the levodopa plasma elimination half-life and improve oral drug delivery. Unfortunately, such formulations did not really improve ‘‘off’’ problems in patients with established fluctuations (Pezoli et al. 1988). Moreover, the early use of the CR versus standard formulations failed to

reduce the incidence of motor complications in the long term. It is likely that these disappointing results were related to insufficient improvements in levodopa pharmacokinetics.

Primarily L-DOPA is absorbed throughout the intestinal tract, where it competes with a range of organic molecules such as peptides and amino acids for uptake and transport. Therefore, Parkinson's disease patients are required to control their diet so as to optimize the L-DOPA absorption (Crevoisier et al. 2003).

Whilst clinicians acknowledge that L-DOPA does inevitably lead to dyskinesia so should be avoided as first line treatment in early Parkinson's disease, it still offers the greatest therapeutic advantage. For this reason, in recent times the development of alternative L-DOPA therapies such as enteral administration of L-DOPA/carbidopa gel (Duodopa™) and orally disintegrating L-DOPA/ carbidopa tablets (Parcopa™) have been developed and launched.

1.5.8 Dopa-decarboxylase inhibition and its partnership with L-DOPA

The vitamin B6 dependent enzyme, dopa-decarboxylase, was first investigated in the 1930's (HOLTZ et al. 1956; HOLTZ 1959; Palm et al. 1967) as it plays a role in many central and peripheral activities. Dopa-decarboxylase is a common name for the enzyme L-aromatic amino acid decarboxylase, and it is ubiquitously distributed with high levels found in peripheral tissues, such as the gut, liver and kidney. The most commonly

used DDCIs are carbidopa and benserazide, which are both hydrazine derivatives that differ only in the introduction of a 2'-hydroxy group into the phenol ring. (figure 1.3). The introduction of decarboxylase inhibitors more than 40 years ago revolutionized the treatment of Parkinson's disease, by reducing side effects and increasing symptomatic relief. However, basic information on their actions is not available and their use in treating Parkinson's disease has not been fully explored.

In 1986, Boomsma showed that the timing of administration of DDCI was important and effected the variability that occurs with carbidopa and benserazide to inhibit the metabolism of L-DOPA, (Boomsma et al. 1986). Interestingly, this difference in DDCI efficacy between carbidopa and benserazide was not apparent with regard to L-dopa response in the clinic. Patients on Sinemet® with a ratio of L-DOPA to carbidopa of 1:10 and Madopar with a ratio of L-DOPA to benserazide of 1:4 had similar responses and alleviation of symptoms.

Both benserazide and carbidopa are non-specific irreversible inhibitors of dopa-decarboxylase and are similar in structure to some anti-depressants like phenelzine (an MAO-A/B inhibitor) (Neff et al. 1974). Despite having similar structures, these molecules, which are able to influence the activity of mono amine oxidase (MAO), it has always been assumed that they have little activity in inhibiting MAO which again highlights that the pharmacokinetics of carbidopa and benserazide have been poorly examined based on literature searches. Despite this, our labs have shown that carbidopa and benserazide are MAO inhibitors (Treseder et al. 2003).

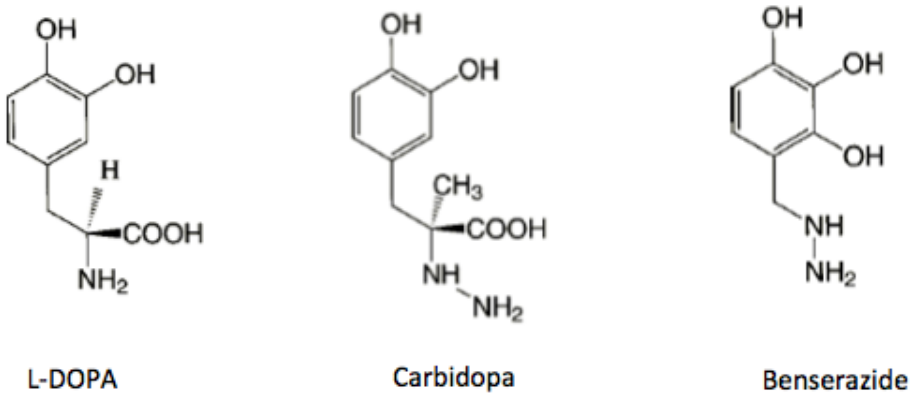


Figure 1.3 Chemical structures of L-DOPA, carbidopa and benserazide (adapted from Da Prada et al. 1987).

Early clinical studies (Boudin et al. 1974) showed that a ratio of 1 part carbidopa or benserazide to 4 parts L-DOPA provided an effective dose ratio to have a therapeutic benefit, although carbidopa was thought to have the best ratio between L-DOPA and dopamine in the urine. The ratio of carbidopa and benserazide to L-DOPA changed over time and through randomised clinical trials resulted in the development of Sinemet® (1:10) and Madopar® (1:4). Clinical trials were then conducted by substituting the DDCI from benserazide to carbidopa (Korten et al. 1975). There was deemed to be no significant difference between benserazide and carbidopa when given in combination with L-DOPA. Both had similar therapeutic effects, side effects, and effects on blood pressure, heart rate, supine and standing. This study indicated that despite the difference between DDCI compositions, there was sufficient inhibition of dopa-decarboxylase to allow the therapeutic benefits of L-DOPA. The study showed that patients did not witness a decline or improvement in there anti-parkinsonian

medication. However, the differences began to arise at the low end of the dose regimen.

The formulation of the Sinemet tablet allowed patients to break the pill into 2 and take them at separate time points avoiding certain dyskinesia episodes. This however, was not the same with Madopar which prevented the capsule from being tampered with, resulting in the patients experiencing more dyskinesia (Greenacre et al. 1976; Reid et al. 1976).

The primary differences between the two dopa-decarboxylase inhibitors is that benserazide needs to be converted into its active metabolite to have an inhibitory effect whereas carbidopa acts directly, not requiring metabolism to an active moiety. Early clinical studies used to assess the effect of DDCIs were carried out comparing L-DOPA alone to L-DOPA combined with carbidopa. These studies only confirmed what we knew about the adverse side effects of L-DOPA when administered alone compared to concomitant treatment with DDCI but they did highlight another interesting but forgotten point. It has been reported in more than one study that patients that received L-DOPA alone reported less dyskinesia than when L-DOPA was combined with DDCI (Greenacre et al. 1976) but this could have been because the plasma levels were too low. This was ignored as the combined L-DOPA plus DDCI was more therapeutic than L-DOPA alone. This meant there was a viable treatment option, which for a time could reverse the Parkinson's disease like symptoms. This is not surprising but does ask the question of whether there is a better DDCI, which could maintain the

therapeutic effects of L-DOPA without inducing dyskinesia expression to the same degree as conventional DDCIs?

1.5.8.1 Carbidopa (MK-486)

Carbidopa (chemical name: L- (-)-α-hydrazino-3,4-dihydroxy-α-methylhydrocinnamic acid monohydrate) is considered to be an irreversible inhibitor of DDC although the evidence available in the literature shows no direct interaction with the enzyme. Rather it appears to scavenge pyridoxal phosphate, an essential co-factor for enzyme activity (Bartholini et al. 1968). This is shown by the ability of additional pyridoxal phosphate to restore decarboxylase activity in both *in-vitro* tissue preparation and in rats treated with carbidopa (Wurtman et al. 1978). Carbidopa does not appear to alter the decarboxylation of endogenous L-DOPA, since alone it has no effect on circulating levels of dopamine or its metabolites (Vickers et al. 1974). Rather, its effects are restricted to blocking the decarboxylation of exogenous L-DOPA, although mechanistically this is difficult to understand.

Carbidopa is rapidly absorbed following oral administration with approximately 40-70% being absorbed in man, monkey or dog although absorption is less marked in the rat (Vickers et al. 1974). The rate of carbidopa absorption is slower than that of L-DOPA and the marked variation in its Cmax suggests a possible reason for L-DOPA dose failure when the two compounds are administered simultaneously (Durso et al. 2000). Maximal plasma carbidopa levels are achieved after 0.5-5 hours in

patients with Parkinson's disease and between 2-4 hours following oral administration in healthy volunteers. Plasma levels following administration of a 50mg dose are in the region of 0.2-0.4μg/ml. The plasma half-life of carbidopa is about 2-3 hours with only a small variation between species.

Once absorbed, carbidopa is rapidly localized to the kidney, liver, lungs and heart (Schwartz et al. 1974). Carbidopa's actions increase intestinal absorption of dietary amino acids including L-DOPA and facilitates L-DOPA's passage through the blood brain barrier in to the brain (Bakke et al. 1974). Determination of total radioactivity over 24 hours following administration of ^{14}C-carbidopa showed that compared to plasma, levels in the kidneys and liver were high while in the heart and in particular the brain lower levels were formed. Maximal levels occurred between the 2-6 hours after administration and steady state levels were achieved after 12 hours (Vickers et al. 1974). At early time points after its administration, carbidopa is excreted unchanged in the urine but at later time points only metabolites are present (Schwartz et al. 1974). Urinary excretion of carbidopa is complete within 7 hours and represents 30% of total urinary radioactivity. Faecal excretion of carbidopa also occurs and accounts for 41-55% of the drug. It is unclear whether carbidopa in the faeces is drug that has failed to be absorbed or drug that has been excreted through the bile. The metabolism of carbidopa involves side chain degradation to produce α-methyl-3-(3-methoxy-4-hydroxyphenyl) propionic acid and 3,4-dihydroxyphenylacetone, which are excreted in urine as glucuronide conjugates. The formation of these metabolites requires O-methylation

and/or removal of the hydrazine group. Decarboxylation of carbidopa is negligible with only 2.8% of the drug being excreted as 14C-CO2 through the removal of the labeled carboxylic acid group. This suggests that it is not itself a substrate for the decarboxylase enzyme. The route of metabolism and excretion of carbidopa is not different in patients with Parkinson's disease compared to healthy volunteers

Following administration of ^{14}C-labelled drug, only small amounts of radioactivity are found in the brain but it is not known whether this was parent drug or metabolites. Immediately following administration of carbidopa, there is inhibition of decarboxylase activity in the brain showing some limited penetration at early time points and at high doses (Da et al. 1987). Carbidopa is relatively specific for inhibiting decarboxylase activity. It does not inhibit COMT but can have inhibitory effects on MAO-B at least in-vitro (Treseder et al. 2003).

Toxicological studies using carbidopa showed low doses resulted in weight gain in dogs but that high doses lead to weight loss. The LD50 for carbidopa following oral administration in the mouse and rat was 1750mg/kg and 5470mg/kg respectively. Since carbidopa is used typically at doses below 20mg/kg this suggests that there is a large window of safety and for therapeutic benefit. Formation of hydrazine molecules as a metabolite of carbidopa has been linked to the occurrence of anemia in the rat, pig and rabbit, and to neuropathological damage in the dog and mouse, and liver damage in the mouse, rat and dog as well as renal changes. However, such potential effects have not been demonstrated in chronic long term studies

in animals (Torchiana et al. 1973).

Early clinical studies in Parkinson's disease highlighted that a combination of L-DOPA with carbidopa reduced the incidence of nausea and vomiting and lead to a more rapid introduction of the drug and dose reduction of L-DOPA leading to improved anti-parkinsonian activity. Other improvements included a reduced risk of cardiac arrhythmias and reduced severity of postural hypotension (Marsden et al. 1973).

1.5.8.2 Benserazide (Ro 4-4602)

Benserazide (DL-Serine 2 - (2,3,4-trihydroxybenzyl) hydrazine) is thought to be an irreversible inhibitor of DDC although the evidence available in the literature shows no direct interaction with the enzyme, rather, like carbidopa, it appears to also scavenge pyridoxal phosphate, an essential co-factor for enzyme activity (Pinder et al. 1976). Although known as a potent inhibitor of peripheral decarboxylase, its clinical pharmacology has only been sparsely investigated. Often benserazide is described as a 'pseudo irreversible' inhibitor of DDC (Burkard et al. 1962; Burkard et al. 1963; Pletscher et al. 1963). This occurs when substrate and inhibitor compete for the enzyme, increasing pyridoxal phosphate and reducing DDC activity. This could result from the hydrazine moiety not reacting with the aldehyde group of free pyridoxal phosphate but only with pyridoxal phosphate once it had bound to the enzyme.

The main reason for these lack of data is due to the complex analysis of the activities of benserazide and/or its active metabolite and the absence of a suitable direct marker of decarboxylase activity (Pinder et al. 1976; Dingemanse et al. 1997). Benserazide can act directly to inhibit decarboxylase activity but may also act through the production of an active metabolite.

Benserazide has a half life of approximately 2 hours, which is lower than carbidopa which is 2-3 hours (Da et al. 1987), but it is not clear whether this refers to the half life of benserazide or its active metabolite or both. Pre-incubation *invitro* with benserazide inhibits DDC activity to a greater extent than when it is added to incubations immediately prior to initiation of the enzyme reaction on addition of substrate. This is probably due to its hydrolysis to the free hydrazone (Ro 4-5127) which inhibits DDC independently of pre-incubation (Burkard et al. 1962). Indeed benserazide works by being metabolized into an active metabolite while being hydrolyzed before or during its intestinal absorption.

The active metabolite of benserazide is tri-hydroxybenzyl-hydrazine or Ro 4-5127 (Bartholini et al. 1975). This is a process that occurs after *in-vivo* administration of benserazide but often it is difficult to detect in *in-vitro* studies (Bondiolotti et al. 1995). This formation of tri-hydroxybenzyl-hydrazine is pH mediated with gastric degradation occurring at gastric (pH ~1) but not at the pH of intestinal contents (~ pH 7). However, the metabolism of benserazide is still not completely understood, (Schwartz et al. 1974) neither *invitro* or *invivo*. Benserazide is initially excreted rapidly

and unchanged in the urine, after which only so far unidentified metabolites have been shown to be present (Vickers et al. 1974). Kinetic analysis is complicated as neither the absorption nor elimination of benserazide follows first order kinetics.

After oral administration of benserazide to Parkinson's disease patients, approximately 66-74% is absorbed from the gut (Schwartz et al. 1974). The incomplete absorption of benserazide is due to its rapid oxidation to quinone-like molecules at the pH of intestinal fluid. Absorption is rapid and thought to occur from the upper small intestine (Pinder et al. 1976). Determination of total radioactivity over 24 hours following administration of ^{14}C-benserazide showed accumulation in the kidneys and liver with lower levels in the heart. Brain levels were low and always below 6% of plasma concentration. Maximal plasma levels of benserazide in man were 1.2-1.3µg/ml and within 1 hour these fell to 0.4µg/ml after 4 hours.

Urinary excretion of benserazide is essentially complete after 6 hours although some metabolites are still present up to 72 hours later. Total urinary excretion for benserazide derived radioactivity over 6-7 days ranged from 60-90% of which 85% was excreted in the first 12 hours (Schwartz et al. 1974), ^{14}C- benserazide was found in faeces for up to 6 days after oral administration and overall. Faecal excretion was between 10-30% (Pinder et al. 1976). It is unclear from the literature whether this represents benserazide or its metabolites or whether they originate from poor absorption or entero-hepatic biliary excretion.

Four hours after oral administration of a range of doses of benserazide to rats, peripheral and central inhibition of DDC in the liver was 40%-95% and in the rat kidney was 18%- 87% over a period of 16 hours. In the brain inhibition ranged from 5% - 70% highlighting that particularly at high doses (over 50mg/kg) benserazide is able to penetrate into the brain (Da Prada et al. 1987). Only in 2001 was the first study looking specifically at the PK profile of benserazide and its active metabolite (Ro 4-5127) undertaken (Grange et al. 2001). The study showed that peak levels of Ro 4-5127 were higher than those of benserazide although DDC activity is concentrated in the gut, kidney and liver, it is the effect of benserazide on the gut wall that is considered to form the most substantial contribution to its activity as first pass metabolism occurs significantly (Iwamoto et al. 1987).

Toxicological studies conducted on benserazide showed that it was similar to L-DOPA, except that benserazide was more toxic to rat skeletal formation during chronic high dose studies. Even at intermediate doses, benserazide exposure still produced toxic effects on the skeletal structure of rats (Rauws et al. 1982). The LD50 value for benserazide ranged from 1750-5610mg/kg in a variety of animal species.

Benserazide administration results in a dose-dependent increase in plasma L-DOPA levels. Even at 200mg three times a day, there was no indication that a maximal response to benserazide had been achieved. L-DOPA levels in patients treated with 50-200mg benserazide three times daily were at

their lowest 6 hours after the initial morning dose of benserazide. This suggested that at these doses, benserazide does not constantly produce decarboxylase inhibition. It adds weight to the argument that benserazides mechanism of action is not truly irreversible (Magnussen et al. 1981).

1.5.8.3 Comparison of benserazide and carbidopa

The pharmacokinetic profile of benserazide in the rat shows that it is more potent than carbidopa. Relatively low doses of benserazide up to 6μmol/kg showed no inhibition of brain DDC activity but still produced between 40-50% inhibition of DDC in the liver and kidney. However to achieve the same levels of inhibition in the liver and kidney with carbidopa, a dose of over 300μmol/kg was required and at these doses brain inhibition of DDC was around 25% (Da et al. 1987a; Da et al. 1987b). This PK profile was similar in the mouse but maximal inhibition was reached at a lower dose of 300μmol/kg for benserazide whereas with carbidopa at 1000μmol/kg dose, inhibition of DDC was only 70%.

Examining the time course profile of benserazide (8.8mg/kg, p.o.) there is a distinctive difference between the high levels of inhibition in the liver and kidney and the small fraction that causes inhibition in the brain (below 10%). Carbidopa (73.3mg/kg, p.o.) produces most inhibition of DDC in the kidney (80%) then liver (50%) and finally in the brain (30%). This data indicates that benserazide is the more potent and therapeutically beneficial DDCI in conjunction with L-DOPA to treat Parkinson's disease patients. Ex-vivo experiments in rats showed that 1 hour after administration of L-

DOPA following a 30 minute pre- treatment with benserazide lead to the highest levels of dopamine in the striatum and plasma compared to carbidopa (Da et al. 1987). These studies over time have highlighted how important it is to get the right dose of DDCI when conducting studies of this nature (Jonkers et al. 2001).

Urinary excretion of carbidopa was complete after 7 hours whereas benserazide was still present in the urine at very low concentration up to 72 hours after dosing. Benserazide prevents L-DOPA metabolism by two routes whereas carbidopa only prevents decarboxylation, benserazide also prevents to a certain degree 3-O-methylation via COMT. This is reflected by causing higher plasma L-DOPA levels when administered with benserazide compared to carbidopa which is a poor substrate for COMT (Hagan et al. 1980). However, the draw back to benserazide being a substrate for COMT is that after benserazide metabolites are produced via COMT, these methylated derivatives may not inhibit dopa-decarboxylase. This can be seen by an initially higher peak L-DOPA concentration after Madopar administration compared to Sinemet however, its effects decline more rapidly (Lieberman et al. 1975; Lieberman et al. 1978).

Both carbidopa and benserazide have no effect if taken without L-DOPA. The potential side effects of these drugs are rare and hold no greater safety issues compared to the aspirin. However, due to the enzyme blocking activity of the drugs, there are reports that they could in high enough doses cause side effects such as hypotension due to its peripheral effects on DDC leading to a reduced blood pressure, which can manifest as dizziness or

nausea (Pinder et al. 1976).

Clinical studies have shown that lowering of the L-DOPA dose with benserazide dose can lead to a 'less complete inhibition' of peripheral DDC (Dingemanse et al. 1997) and could possibly avoid some adverse effects of long term L-DOPA therapy as it could result in a reduction of the dose-normalized L-DOPA exposure. Due to differing PK profiles between carbidopa and benserazide and their potencies of DDC inhibition the ratios of the doses now used in clinical trials are being investigated.

1.5.9 L-DOPA Pro-drugs

In order to strengthen the pharmacological activity of anti-parkinsonian drugs, enhancing their penetration of the blood-brain barrier (BBB), different approaches are possible. Primarily the prodrug approach currently offers great promise to optimise physicochemical characteristics including optimising peripheral decarboxylase inhibition, enhancing duration of action and allow L-DOPA sparing regimens. These all focus on increasing continuous dopaminergic stimulation. In addition, novel therapeutic strategies based on formulations linking dopaminergic drugs with neuroprotective agents, increasing striatal L-DOPA levels and offering sustained release of the drug without any fluctuation of brain concentration, offer promising avenues for development of other effective new treatments for Parkinson's disease (Sozio et al. 2012).

L-DOPA, the gold standard in Parkinson's disease treatment has warranted the attention of chemists and industry efforts to derive new and improved versions of the molecule. To date none have come close to the same efficacious levels as L-DOPA in a clinical setting.

One scientist who features heavily in L-DOPA pro-drug chemistry is Nicholas Bodor whose work on L-DOPA pro-drugs has been to date the most extensive in the literature (Bodor et al. 1977). Nicholas Bodor has worked extensively on L-DOPA pro-drugs and established that therapeutic effects of L-DOPA are correlated to blood L-DOPA concentrations and that the key to improving the level of L-DOPA in the periphery were changes in water and lipid solubility, transport properties and metabolism (Bodor et al. 1977; Sozio et al. 2012; Ishikura et al. 1995).

There are several key features, which need to be considered in the development of L-DOPA pro-drugs, which include the chemistry protecting the pro-drug and the bioavailability (Bonina et al. 2003). The approach that Bodor had taken was to use acetyl and pivaloyl protective groups for the catechol, methyl / benzyl esters and N-terminal peptides for the carboxy, and formyl and C-terminal dipeptides for the amino group (Bodor et al. 1977). His work clearly showed that the protected di-peptides containing two L-DOPA moieties do result in a greater L-DOPA delivery as determined by L-DOPA to dopamine ratio. Despite this, the metabolic profile of L-DOPA pro-drugs are not different to L-DOPA itself which is primarily due to the extensive metabolism the pro-drug undergoes (Bodor

et al. 1977). This is an important point in all L-DOPA pro-drug formation as racemization of L-DOPA to D-DOPA would significantly reduce the anti-parkinsonian benefits of its administration due to the parent compound being excreted as DOPA and dopamine (Shindo et al. 1973).

Their have to date been several studies using novel L-DOPA pro-drugs to investigate how the absorption sites and overall efficacy vary from standard L-DOPA formulations. One such study was carried out using NB-355 (L-3-(hydroxyl-4-pivaloyloxphenyl) alanine) a variant of L-DOPA, administered into different sites of the gastrointestinal tract. In these studies L-DOPA, or NB-355 were injected into ligated loops of the small intestine and the absorption of the drugs was monitored (Hisaka et al. 1989). This study showed that NB-355 was absorbed from the ileum rather than the duodenum or the jejunum, whereas L-DOPA was rapidly absorbed from the jejunum. The results concluded that NB-355 produced prolonged L-DOPA plasma levels due to its slower absorption and longer transit time through the gut. When NB-355 was tested in MPTP primate models it also showed improved efficacy with a lower incidence of dyskinesia (Tye et al. 1989). However, this did not transfer into the clinical setting, offering no further improvement on standard L-DOPA therapy (Ihara et al. 1989).

The literature regarding L-DOPA pro-drugs, especially assessment of their behavioral effects in experimental models is limited. In recent years, there has been a growing interest in the use of L-DOPA pro-drugs (Di et al. 2011)

with various hypothesis and review articles being published but little has been investigated in a clinical or animal model assessment. The majority of information about these compounds relates to structure and chemistry and is found in patents, which have been submitted for intellectual property rights.

1.5.10 Dopamine agonists

The rationale for the use of dopamine agonists is to delay the initiation of L-DOPA or to decrease the dose of L-DOPA, thereby reducing the motor complications of long-term levodopa therapy. Dopamine agonists, when used alone, rarely promote the development of dyskinesias and motor fluctuations that complicate levodopa treatment (Watts 1997). The benefit of dopamine agonists compared to L-DOPA is that unlike the latter, they do not require conversion to an active metabolite (Wooten 2003). The conversion of L-DOPA into DA requires the presence of some intact dopaminergic neurones (although difficult to quantify especially when the role of other neuromodulators is factored in) as well as other surrounding cells like glia and serotonergic neurones, and where these are not present in sufficient quantities, this conversion may not be able to take place on a scale sufficient to implement its anti-parkinsonian effects.

Without the need for conversion, dopamine agonists act either directly to stimulate striatal dopamine receptors or on the dopaminergic neurones causing endogenous DA release (Wood 2010). The use of DA agonists also

allows patients to have a more normal life in terms of diet and the way they have to arrange their day around the timing of when they take their medication. In addition, DA agonists do not compete with amino acids for absorption in the gut so can be transported across the BBB for therapeutic effect, so permitting them to be administered at any point during the day unlike L-DOPA which needs to be taken with a sufficient time period before/ after food to maximise its therapeutic effects (Wooten 2003). The use of L-DOPA has sparked tests into its toxicity in recent times with the suggestion that L-DOPA can lead to neurotoxic metabolites, which play a part in oxidative stress, which can contribute to the progression of the disease (Dexter et al. 2013). *Invitro* and in animal models, dopamine agonists have shown some protective effect on dopamine neurones (Herrero et al., 2011). Apart from the symptomatic benefit, dopamine agonists may also have a neuroprotective role (Kyriazis 2003) however, a recent study (PROUD) suggested no clinical neuroprotective effect (Schapira et al. 2013). The principle advantage held by most DA agonists over L-DOPA therapy is there longer half life (Olanow et al. 2000).

The ability to sustain dopaminergic stimulation is believed by some to reduce the risk/ onset of dyskinesia. This view point is strengthened by evidence showing that the use of short acting DA agonists such as apomorphine induce similar involuntary movements such as those seen in Parkinson's disease patients on continued L-DOPA treatment (Jenner 2002). DA agonists have the advantage of being selective for certain DA receptors. Most DA agonists like ropinirole and pramipexole are selective

for the D2 family receptors whereas L-DOPA via dopamine is differentially selective and acts on both D1 (low potency) and D2 (high potency) receptors. The concern with L-DOPA here is that burst stimulation of D1 receptors is believed to increase the likelihood of dyskinesia (Wooten 2003).

Direct-acting dopamine agonists have been available for the treatment of Parkinson's disease for a number of years. Originally introduced as longer-acting adjuncts to L-DOPA, these compounds have been increasingly used as monotherapies for early symptomatic treatment for Parkinson's disease. Pramipexole, ropinirole and rotigotine have all been shown to provide improvement in parkinsonian disability in Parkinson's disease compared to placebo, and bromocriptine appears to be similarly efficacious (Rinne et al. 1998; Rascol et al. 2002; Goetz et al. 2005).

However, the therapeutic profile of dopamine agonism in Parkinson's disease is far from ideal. Symptomatic relief is compromised by dopamine agonist therapy compared to that obtained with L-DOPA, and within 3 years 50% of patients receiving dopamine agonist monotherapy require L-DOPA supplementation, with the number rising to 70% within 5 years (Lees et al. 2001; Olanow 2002). The principal rationale for the use of dopamine agonists is to delay the problem of dyskinesia. However, although dopamine agonists do induce significantly less dyskinesia than L-DOPA, they are not free of this problem. Short-acting dopamine agonists, such as apomorphine, with similar half-lives to L-DOPA induce marked dyskinesia when administered in a pulsatile manner in primates (Jenner

2002). Furthermore, once priming for dyskinesia with L-DOPA has occurred, dopamine agonists will induce the same involuntary movements observed with L-DOPA administration (Jenner 2002).

Dopamine agonists have a greater proclivity to induce adverse effects other than dyskinesia, such as uncontrollable somnolence, dizziness, hallucinations and nausea (Olanow 2002; Avorn et al. 2005). Furthermore, ergot-derived dopamine agonists, particularly pergolide, have recently been linked to potentially fatal fibrotic syndromes (Van et al. 2004; Tintner et al. 2005). Current evidence suggests that non-ergot derived agonists are not subject to the same problems, but this is still a concern (Tintner et al. 2005; Goetz et al. 2005b). A final disadvantage is that dopamine agonist therapy is considerably more expensive than L-DOPA, a factor that is also likely to be important in the prescription policy of some clinics.

One of the most common problems with dopamine agonists in the clinic is impulse control disorders (ICDs). Impulse control disorders are common in Parkinson's disease, occurring in 1 in 10 patients (Hallet et al. 2012). Patients with impulse control disorder make more risky choices in the 'Gain' relative to the 'Loss' condition. In patients with impulse control disorder, dopamine agonists were associated with enhanced sensitivity to risk (would rather try and win money rather than save money) along with decreased ventral striatal activity again with the opposite in Parkinson's disease controls. Patients with impulse control disorder appear to have a bias towards risky choices independent of the effect of loss aversion. Dopamine agonists enhance sensitivity to risk in patients with impulse

control disorder possibly by impairing risk evaluation in the striatum. This has led to gambling problems, hypersexual activity and other compulsive activities. Clinicians face a tough challenge in achieving a right therapeutic balance which between ICD and sub-therapeutic doses. (Voon et al. 2011).

In summary, initial treatment with dopamine agonists is a valuable option in stalling the motor complications of L-DOPA in Parkinson's disease patients. This is particularly useful in cases such as autosomal-recessive juvenile Parkinsonism, where patients are especially susceptible to developing dyskinesia, and would have to live with these increasingly severe complications for many years (Khan et al. 2003). However, dopamine agonists and other alternatives are not in a position to replace L-DOPA as the drug of first choice for this condition, and therefore it is still essential to develop alternative therapies, or adjunct therapies to improve the side-effect profile of L-DOPA.

1.6 L-DOPA therapeutic problems

Repeated and chronic use of L-DOPA both for early and advanced cases of Parkinson's disease has its drawbacks. The primary complication and the one, which draws in the largest focus from the research community, is dyskinesia. Dyskinesia is abnormal uncontrolled movements, which takes the form of chorea (dance like movements) or dystonia (rigid muscle tension). Dyskinesias can prevent patients from leading a normal life and

often interfere with the quality of daily living by stopping patients from carrying out routine activities. Dyskinesia appear in over half of all patients within 5 years after commencing L-DOPA therapy (Ahlskog 2003). Furthermore, chronic L-DOPA therapy leads to a problem known as 'wearing off'. This is the phenomenon whereby 'on' time (good therapeutic benefit is experienced) is decreased and there is an increase in 'off' periods (loss of therapeutic benefit). Hence, potential adjunct therapies and advancements in L-DOPA therapy optimization that can reduce L-DOPA induced dyskinesia (LIDs) are of the greatest importance.

1.6.1 L-DOPA–induced dyskinesias (LIDs) – physiology

The mechanisms that give rise to the developments of dyskinesia are not well understood and are subject to ongoing investigation. There are various factors associated with the induction of LIDs. Preclinical and clinical observations provide evidence that the development of LID requires nigrostriatal dopaminergic denervation, intact postsynaptic basal ganglia neurons, and exogenous administration of levodopa (Di Monte et al. 2000).

LID was first reported by Cotzias et al, the group credited with the first successful use of L-DOPA in treating Parkinson's disease. Subsequent reports highlighted their high incidence, varied phenomenology, and treatment-limiting effect. Initially thought to be associated only with the peak plasma levels of levodopa, later reports of diphasic dyskinesias and early morning dystonia emphasised a rather complex picture of LID.

It is known that dyskinesias appear only after dopaminergic therapy and there is a time lag between the start of treatment and the emergence of LID (Cotzias et al. 1969).

It is suggested that pulsatile (as opposed to a continuous, physiological) stimulation of the postsynaptic receptors due to intermittent administration of levodopa leads to downstream changes in proteins and genes, causing alterations in striatal output in a way that promotes dyskinesias. Disinhibition of the primary and associated motor cortex secondary to increased outflow may account for LID.
It has been hypothesized for some time that the pulsatile nature of dopamine release elicited by chronic L-DOPA therapy might be responsible. This leads to abnormal stimulation of denervated striatal receptors that are stimulated tonically under physiological conditions (Horowski 1988; Horowski et al. 1988; Chase et al. 1989). This is borne out by the fact that long-acting dopamine agonists, such as pramipexole and ropinirole, delay onset and reduce severity of motor complications when used as therapy in early Parkinson's disease (Rascol et al. 2000). Other factors of influence can be the dosing regimen itself, i.e. the number of times a day a patient takes their medications but also the abnormal conversion of L-DOPA to dopamine by serotonergic neurons.

The induction of dyskinesias by L-DOPA involves a priming mechanism, and subsequent use of a dopamine agonists or L-DOPA will induce dyskinetic episodes. Once dyskinesias have been primed for, the

introduction of a long-term drug holiday or different dopaminergic treatment will still result in the manifestation of dyskinesia once drug treatment re-starts.

Below is a flow of events which has been postulated for the development of LIDs:

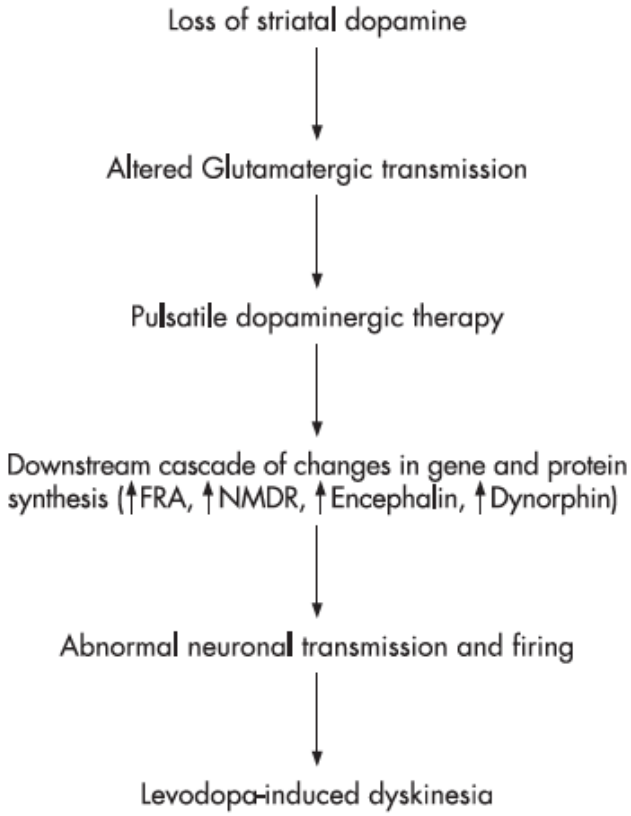


Figure 1.4. Hypothesised flow diagram of LID induction (adapted from Thanvi et al. 2007)

Motor complications are most commonly observed in patients on L-DOPA therapy and are less common in patients taking other antiparkinsonian medications. Hence, current views suggest that aberrant dopamine metabolism has a prominent role in L-DOPA-induced motor fluctuations

(Rascol et al. 2000). Indeed, the notion that the capacity of the nigrostriatal terminals to synthesize and store dopamine diminishes with disease progression suggests a presynaptic mechanism underlying the generation of motor fluctuations. However, several reports have indicated that molecular changes occurring at dopamine receptors and their downstream signaling targets during dyskinesia may be responsible for the modifications ultimately leading to the occurrence of motor complications in Parkinson's disease. For example, increased dopamine D1 receptor sensitivity and downstream signaling have been reported in primates with L-DOPA-induced dyskinesia (Aubert et al. 2005).

Chronic L-DOPA treatment has been shown to cause profound changes in striatal glutamatergic signaling. NMDA receptor subunits undergo profound adaptive modifications (Hurley et al. 2005), with an abnormal redistribution of the NR2B subunit between synaptic and extra synaptic membranes.

The details of changes in genes and proteins caused by abnormalities in the dopaminergic and non-dopaminergic transmission eventually leading to LID are beginning to be clear. Fos- and Fos-related proteins (FRA) are induced in the striatal neurons by excessive glutamatergic inputs (caused by loss of striatal dopamine) (Calon et al. 2000).

Current opinion on dyskinesia induction suggests that it is dependent on the nature of the dopaminergic stimulation involved, with continuous

stimulation producing a lower incidence of dyskinesia (Obeso et al., 1994;Jenner. 2004). This is proposed to create a constant level of postsynaptic dopamine receptor stimulation, and thus a physiological pattern of striatal and basal ganglia output activity. This is supported by evidence from the clinic (Olanow. 2002), and in MPTP-treated primates (Pearce et al., 1998; Smith et al., 2005).

Increasing evidence suggests that the manifestation of dyskinesia depends on abnormal storage of information, such as in corticostriatal long-term potentiation (LTP) and depression (LTD). Indeed, compromised synaptic depotentiation, an important aspect of LTP regulation, at corticostriatal synapses was linked to dyskinesia in a study in rats (Picconi et al., 2003). This was in turn linked to increases in the striatal levels of the phosphorylated form of the 32kDa dopamine- and cAMP-regulated phosphoprotein (DARPP-32), a potent protein phosphatase inhibitor linked to dopamine D1 and adenosine A2A receptor activation (Picconi et al., 2003;Hakansson et al., 2004). The persistent changes that result from priming appear to involve enhanced sensitivity of NMDA-type glutamate receptors (Calabresi et al., 2000), and alterations in these receptors with respect to LID are the subject of much current investigation (Hurley et al., 2005).

It is postulated that the expression of dyskinesia results from a set of circumstances opposite to those in the parkinsonian state, i.e. increased direct striatal inhibition and a hypoactive STN leading to reduced GPi/SNr output activity and disinhibition of the thalamocortical pathway.

There is good evidence to suggest that the GPi is hypoactive in primates and patients with dyskinesia. Problems arise when one considers that pallidotomy also improves dyskinesia. As with the parkinsonian state, it has been proposed that aberrant neuronal firing patterns and their transfer throughout the motor circuitry cause dyskinesia. Further, the differences in the variety of dyskinesias that exist (Vidailhet et al., 1999) have been proposed to arise from a varying balance of activity in the pathways involved and a multitude of neuronal electrophysiological characteristics within these pathways.

In addition to their short-term actions, mediated by ion channels or second messenger systems, it is now clear that neurotransmitters can induce long-term (over the course of hours & days) changes in the central nervous system (Picconi et al. 2003). These actions are proposed to underlie learning and plastic responses that are involved in the development of long-term behavioural phenomena such as LIDs.

Constitutive immediate-early gene (IEG) expression in unstimulated cells is typically low, but can be rapidly induced (within minutes) in a transient manner by neurotransmitter activation of certain receptors. This is thought to occur via phosphorylation of transcription factors such as cyclic AMP response element binding protein (CREB) and activator protein-1 (AP-1) by protein kinases (Andersson et al., 2001). IEGs are translated in the cytoplasm; the resultant gene products are predominantly transcription factors that are thought to stimulate the subsequent transcription of late response genes several hours later. These late response genes encode proteins that mediate the physiological response and have been shown in

animal models to increase dyskinesia expression (Cenci et al. 1999; Cenci et al. 2006).

In summary, neuronal transmission is altered in the dyskinetic state and future treatments that are of interest in the treatment of dyskinesias are likely to restore striatal neurotransmission to normal levels.

1.7 Hypothesis

Parkinson's disease therapy is still focused on symptomatic treatment for the main clinical features. Even after 40 years since the introduction of L-3,4-dihydroxyphenylalanine (L-DOPA) as the 'gold' standard in Parkinson's disease treatment, long-term use of L-DOPA is known to result in motor complications (dyskinesia), wearing-off, 'on-off' effect and a progressive reduction in clinical efficacy.

Years of intensive research exploring new pharmacologic agents and treatments options have failed to deliver a more effective treatment than L-DOPA. For this reason, in recent years drug discovery has begun to re-focus on whether there is more that we can do with L-DOPA to improve clinical efficacy and patient outcomes.

The hypothesis for this thesis was:

L-DOPA, the current 'gold standard' pharmacotherapy for Parkinson's disease can be improved by optimising L-DOPA treatment strategies

1.8 Aims of this thesis

In order to test this hypothesis, this thesis aimed to investigate whether changes in the way L-DOPA treatment strategies are administered could lead to improved responses in animal models of Parkinson's disease. Specifically the following aims were addressed:

1) To potentiate the clinical response of L-DOPA by maximising the efficiency of peripheral dopa decarboxylase (DDC) inhibition

2) To enhance the clinical response of L-DOPA through prodrug delivery

3) To optimise L-DOPA's clinical therapeutic window through combination therapy with dopamine agonists

Chapter 2

General Methodology

2. General Methodology

The aim of the studies reported in this thesis was to investigate ways in which L-DOPA treatment of Parkinson's disease patients could be improved. In particular the following aims were addressed:

- The relationship between dose and timing of administration of DDCIs and L-DOPA's effect on motor function in 6-OHDA lesioned rats and in the MPTP-treated common marmoset
- The effectiveness of PRX 1354, a pro-drug of L-DOPA, on the reversal of motor deficits and expression of dyskinesia in MPTP-treated common marmosets
- The ability of L-DOPA in combination with the dopamine agonist, pramipexole, to control motor function and reduce dyskinesia expression compared to either drug given alone

In order to achieve these aims, behavioural studies were performed in 2 animal models of Parkinson's disease. The general methodologies for the production of animal models and their behavioural assessment are described in this chapter.

2.1 Toxin based research models into Parkinson's disease

Neurodegenerative diseases are manifestations of abnormal changes in complex behaviour integrated in our neurological systems. Animal models are therefore vital tools in our understanding of their pathologies, and the effects of drug treatment on these pathologies. Although animal models of

Parkinson's disease can't replicate all of the idiosyncrasies of the human condition they have proven useful in advancing general understanding of the basal ganglia system and its role in movement execution.

The destruction of the nigrostriatal system seen in Parkinson's disease is replicated in the two most commonly used and best-characterised models of the disorder, the MPTP-treated non-human primate and the unilateral 6-hydroxy dopamine (6-OHDA) lesioned rat. These toxin-induced models are used in the studies described in this thesis, and are described below in detail.

2.2 Unilaterally 6-hydroxydopamine–lesioned rat

Since the early 1960's it has been established that 6-hydroxydopamine (6-OHDA) is capable of depleting peripheral organs of noradrenaline (WEINER et al. 1962; PORTER et al. 1963) but in 1968 Ungerstedt showed that 6-OHDA was able to induce degeneration of central monoamine neurons (Ungerstedt 1968). This was able to offer a simple but vital method of tracing these neuronal systems in the brain by studying the accumulation and disappearance of the neurotransmitter after local injections of 6-OHDA. The degeneration of the nigro-neo striatal system induced by 6-OHDA produced major disturbances in motor function. Since then, this has been a vital method for assessing dopaminergic activity, manipulation and the influence of CNS type drugs in Parkinson's disease (Ungerstedt 1968).

6-OHDA is injected via stereotaxic surgery into one side of the rat brain to produce a lesion greater than 95% in the nigro striatal dopaminergic tract. 6-OHDA is an analogue of dopamine, utilised to create the most commonly used animal model for Parkinson's disease, the unilateral 6-OHDA-lesioned rat model. This animal model was created in the 1960s by Ungerstedt (Ungerstedt 1968), using stereotaxic surgery to deliver this neurotoxin, which causes selective degeneration of catecholaminergic neurones. The unilateral 6-OHDA lesioned-rat model of Parkinson's disease, involves a lesion of the nigrostriatal dopaminergic tract, typically via localised injection at the medial forebrain bundle, since the nigrostriatal projection passes through this area and it constitutes a more convenient target than the SNc itself (Figure 2.1).

6-OHDA destroys both noradrenergic and dopaminergic cells equally which means that rats will need to be pretreated with the noradrenaline uptake blocker desipramine (desmethylimipramine) to protects these cells from degeneration (Breese et al. 1971).

The 6-OHDA lesioned rat provides a convenient, chronic rodent model that has proven a remarkably accurate tool for the investigation of mechanisms and treatments of Parkinson's disease (Cenci et al. 2002) and for these reasons has been employed in the work undertaken here.

2.2.1 Neuropathological effects of 6-hydroxydopamine lesion in the rat

Unilateral lesion of the nigrostriatal pathway with 6-OHDA leads to a number of biochemical changes in the dopamine system, resulting in

imbalances in dopaminergic sensitivity between the two hemispheres. Dopamine and tyrosine hydroxylase levels are reduced in the lesioned striatum and SNc (Breese et al. 1971) and changes in striatal postsynaptic dopamine receptor function are observed. This includes the increase in dopamine release and the supersensitivity of receptors. Striatal output pathway peptide mRNAs are altered and changes in striatal postsynaptic dopamine receptor function occur.

Receptor changes are dependent on lesion extent and time after lesion (Araki et al. 1998).

Local 6-OHDA injection into the medial forebrain bundle or substantia nigra induces a selective lesion in the nigrostriatal pathway, while dopaminergic neurones of the mesolimbic system are mostly unaffected. As with MPTP administration, neuronal loss induced by this method of 6-OHDA lesioning is acute, and therefore does not mirror the progressive evolution observed in Parkinson's disease.

2.2.2 Behavioural effects of 6-hydroxydopamine lesion in the rat

Immediately following lesioning, and partially due to the anesthetic effects, rats become adipsic and aphagic, leading to weight loss; however, this is only temporary in the unilateral lesioned model. Rats with an established unilateral 6-OHDA lesion of the nigrostriatal pathway exhibit low-level rotational behaviour ipsilateral to the lesion and exhibit sensory neglect to the side contralateral to the lesion (Ungerstedt et al. 1970).

Dopamine agonists induce asymmetrical motor activation in unilaterally 6-OHDA lesioned rats, resulting in circling activity. The direction of turning is dependent on the side where dopaminergic activity is dominant and is therefore dependent on the mechanism of action of the agent used. Dopamine agonists, such as apomorphine, act on supersensitive postsynaptic dopamine receptors resulting in turning away from the lesion, i.e. contralaterally. Dopamine releasing agents and reuptake inhibitors, such as amphetamine, act on presynaptic terminals, resulting in increased transmission in the intact side of the brain and turning towards the lesion (ipsilateral). The magnitude of rotation is dose dependent. Drug responses are also dependent on lesion size. Response to apomorphine typically requires at least a 90% lesion, whereas amphetamine is able to elicit a response (figure 2.2) with an approximately 50% lesion (Ziegler et al. 1988; Hudson et al. 1993). Patterns of turning behaviour also vary depending on the mechanism of drug action.

2.2.3 6-OHDA lesioned rats and chronic L-DOPA therapy

Behavioural measures in the unilaterally 6-OHDA lesioned rats have primarily been used to investigate the acute dopaminergic effects of pharmacological agents. Quite often the 6-OHDA lesioned rat is the best way to assess dopaminergic activity of any new compounds or molecular entities. Sensitisation of the circling response occurs with repeated administration of these agents, and has been proposed to be indicative of priming for the motor complications that are observed with chronic L-

DOPA therapy (Ziegler et al. 1988; Carey 1991; Carey 1993; Henry et al. 1998).

The duration of circling response decreased in some studies, proposed to be reflective of the 'wearing-off' effect seen in late-stage Parkinson's disease (Papa et al. 1994). Intermittent pauses in circling behaviour have also been reported with repeated L-DOPA administration, and proposed to be reflective of 'on-off' fluctuations (Papa et al. 1994; Mura et al. 2002). However, the manifestation of other behaviours, may be responsible for these interruptions (Mura et al. 2002). The 6-OHDA lesioned rat model has been developed into a more comprehensive animal model with the introduction of the abnormal involuntary movements scale (AIMs) (Cenci et al. 1998). AIMs were separated into four categories: axial, i.e. abnormal posturing or choreiform twisting of the body contralateral to the lesion; limb, i.e. purposeless, repetitive jerky movements of the forelimb contralateral to the lesion, often combined with grabbing movements of the paw; orofacial, i.e. vacuous jaw movements, possibly combined with tongue protrusion and locomotor activity with directional bias. These respond to drugs known to reduce dyskinesia clinically e.g. amantadine, and this model is used as an early screen for anti-dyskinetic drugs (Spinnewyn et al. 2011).

2.2.4 Animals – 6-OHDA lesioned rats

Male Wistar rats weighing between 250-280g (source: Harlan UK) were given one-week acclimatisation after arrival at the site of designation. The animals were typically housed in groups of up to 4, but never singly. Animals were kept on a 12 hour light / dark cycle at approximately 50% humidity and room temperature of ~20°C. Rats had access to Purina rat food pellets and water ad libutum when in the home cage. All experiments were performed in accordance with Home Office regulations under the Animals (Scientific Procedures) Act 1986. The project licence under which the 6-OHDA lesioned rat work was carried out was 90/6019.

2.2.5 Stereotaxic surgery for the unilateral medial forebrain bundle lesion in 6-OHDA lesioned rats

Male Wistar Rats were treated with desipramine (25mg/kg, intra-peritoneal (i.p.), 30 minutes prior to 6-OHDA) to protect noradrenergic terminals. Rats were then anaesthetized in an induction chamber using isoflurane (1-2% in medical oxygen carrier gas), placed in a Kopf stereotaxic frame where anaesthesia was maintained with 0.5-1.0% isoflurane. A sagittal incision is made in the scalp which is equidistant and just caudal to both rat ears and the skin is held back using small surgical retractor clips after which a 2.0mm-diameter hole is made in the skull at coordinates AP: -0.26mm ML: +0.2 mm & DV -0.88mm (Paxinos et al. 1985) 6-OHDA (8 µg free base in 4 µL of 0.9% saline containing 0.05% ascorbic acid) is injected into the left median forebrain bundle at a constant rate

over 4 minutes (1µl/minute) using a 10-µL Hamilton syringe lowered to -8 mm below the dura. The needle remained in place for a further 4 minutes before being removed, and the wound cleaned and sutured using viycryl dissolvable sutures. Rimidyl hydrochloride (2.5 mg/kg, Dunlop's Veterinary Supplies, Dumfries, UK) was administered for pain relief and a rehydration treatment of 5% glucose in 0.9% saline (up to 5ml i.p.) was given prior to recovery from the anaesthetic. Animals were then placed in a heated recovery unit until they regained full consciousness, the wound was double checked to ensure that there was no excessive bleeding or discomfort and the animal returned to home cage.

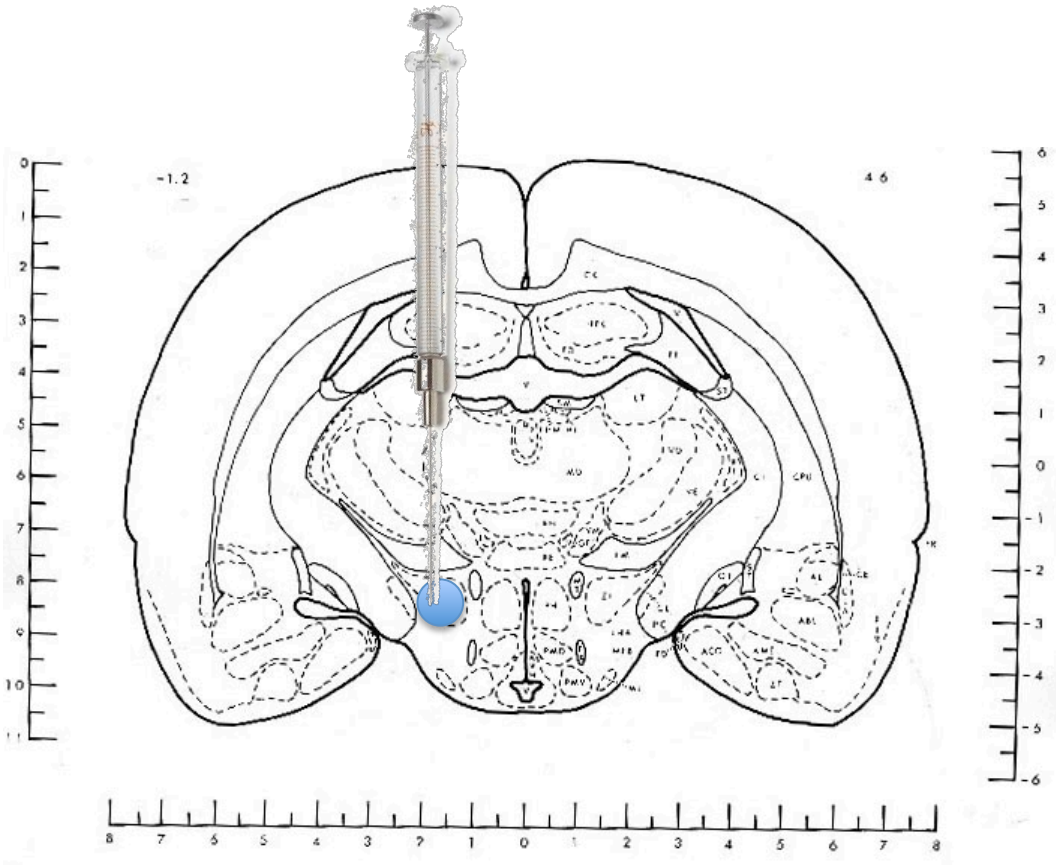


Figure 2. 1 Diagrammatic representation showing the injection of 6-OHDA into the left medial forebrain bundle

Co-ordinates from bregma: -2.6mm AP, +0.2mm ML, -8.8mm DV (from (adapted from Paxinos and Watson.1998). The number in the top left corner specifies the AP coordinate with respect to bregma. The scale along the left side allows you to determine the DV coordinate with respect to bregma. The scale along the bottom of the page is used to determine ML coordinate from bregma or the midline.

2.2.6 Postoperative care for 6-OHDA lesioned rats

With the return of full consciousness and being placed back into the home cage, animals were given additional food in the form of softened Purina rat pellets for the first week after the lesion. All animals were weighed once daily and body weight was managed by either further supplementing diet in the presence of weight loss. If sutures had not dissolved by day 5, they

were removed under light anaesthesia. One week post surgery all additional food supplements and weight monitoring was reduced to three times a week.

2.2.7 Verification of the 6-OHDA lesions

Approximately, 3-4 weeks post surgery, lesions should be stable. In order to confirm that the correct lesion is made without having to sacrifice the animals, they are treated with amphetamine (0.2mg/kg, s.c.) and placed in automated equipment (Rotometers- Roto Rat, source: Med Associates Inc.) to measure rotational activity as discussed in detail later in this chapter. Only those rats that achieved 5 or more turns per minute at peak rotational behavior were used for further study (this forms the criteria for inclusion of the animals in further studies). This corresponds to a lesion causing greater then 90% striatal dopamine depletion (Cenci et al. 2006; Lane et al. 2008). Animals were then assigned into different groups so that each group has a similar median value for rotations for 4 hours. This ensures that the response between groups is of a similar magnitude and will avoid the data being skewed. Animals were then given three days washout and subsequently primed with L-DOPA. These animals have already been included in the study and the L-DOPA priming is to ensure consistent and reproducible L-DOPA induced rotations. This entails all animals being treated with L-DOPA, 12.5mg/kg (plus benserazide 10mg/kg) orally (p.o.) for approximately 8-10 days. This sensitised all animals to L-DOPA as previously described (Carey. 1991) (figure 2.3). At this point animals were

then available for allocation to a specific study. Figure 2.3 A and B show the progressive increase and plateau of rotational responses to L-DOPA between day 1 (almost no turning) through to day 8 (a mean rotational effect of ~750) which was steady from day 6.

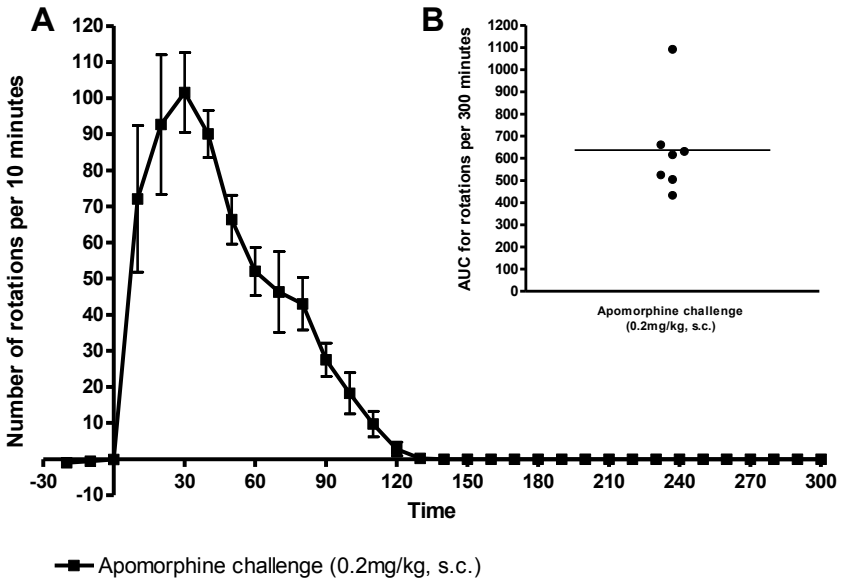


Figure 2. 2 The effect of apomorphine challenge in newly lesioned 6-OHDA rats (inclusion criteria)

Rotational response of 6-OHDA lesioned rats (n=7) after administration of apomorphine, 0.2mg/kg subcutaneously (s.c.). Animals were placed into rotometers for a 30 minutes acclimatization period (-30 minutes to 0 minutes) then treated with apomorphine and rotations monitored for up to 5 hours. Data shown as time course (A) and area under the curve expressed as individual animals with the mean value for the group (B). No statistical analysis was carried out for this data.

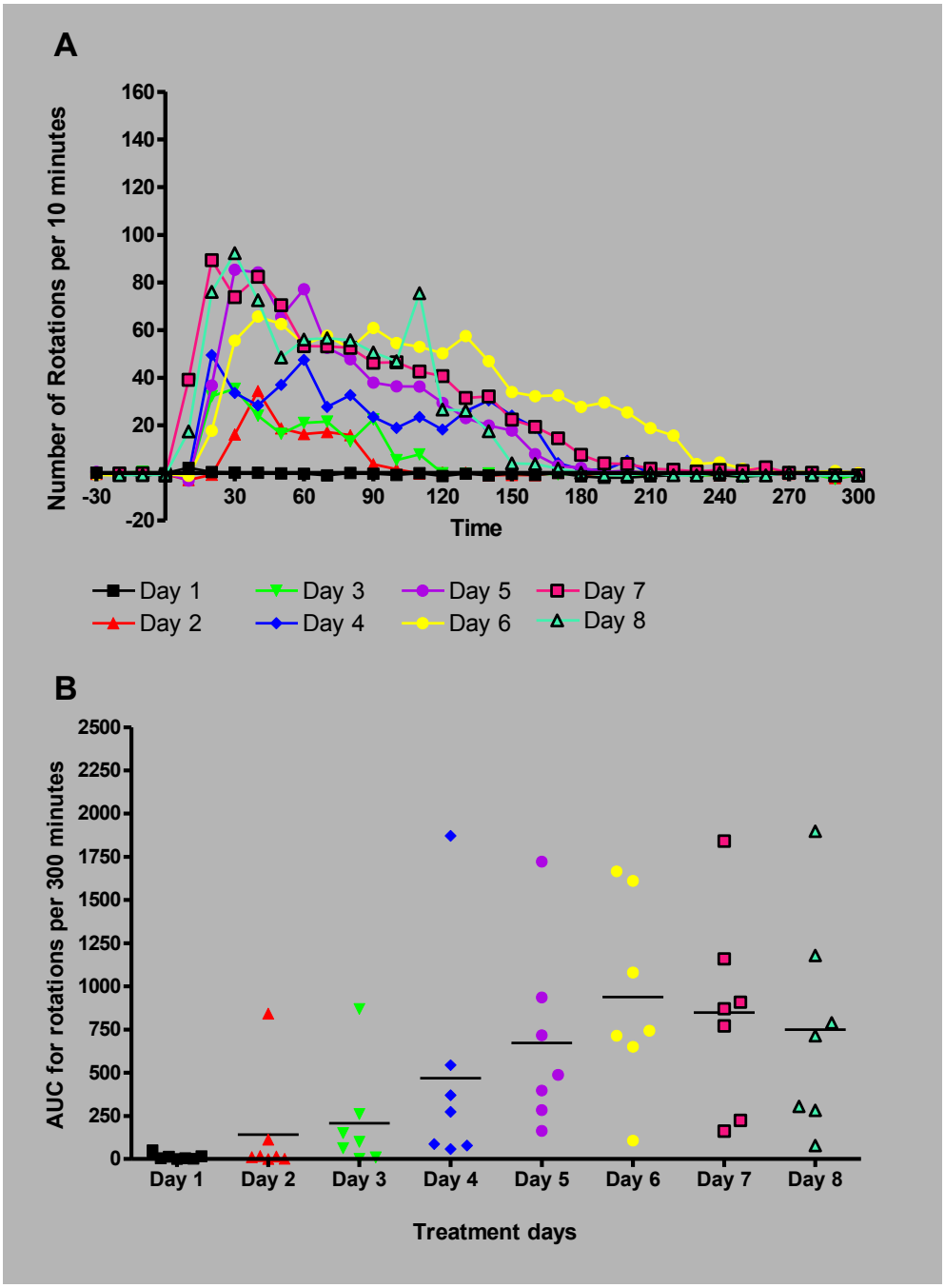


Figure 2. 3 Chronic L-DOPA treatment (priming) in newly lesioned 6-OHDA rats for 8 days (post study inclusion) to ensure consistent and reproducible rotational responses

Rotational response of 6-OHDA lesioned rats (n=7), which had passed inclusion testing via apomorphine challenge. Data shows rotational response following administration of L-DOPA (12.5mg/kg, p.o.) concomitantly with benserazide (10mg/kg, p.o.) over 8 days. All animals were placed into rotometers for a 30-minute acclimatisation period (-30 minutes to 0 minutes) then treated with L-DOPA and DDCI, and rotations monitored for up to 5 hours. All n=7 animals were used for further experiments. Data shown as time course (A) and total area under the curve expressed as individual animals with the mean value for the group (B).

2.2.8 Assessment of rotational behaviour in the 6-OHDA lesioned rat using Roto Rat

Animals were treated with the required drug combination; the rotational response recorded using the Med Associates Inc. Roto Rat hardware and Roto Rat software version 2. 6-OHDA lesioned rats would be removed from the home cages at 'lights on' (approximately 7am). Rats were placed in 'waist coat' type jackets (the forepaws of the rat are placed into the sleeves and the coat is strapped up at the back) (figure 2.4). The jackets were attached to a suspended tether, which was attached to an activity sensor, which detected rotational behavior in 45^{o} units every minute and accumulates these to give a total contralateral or ipsilateral to the lesion rotational count. Rats were then placed into stainless steel bowls surrounded by a clear perspex tube to prevent them from climbing out of the bowl (Figure 2.4A and 2.4B). All statistical analysis was carried out on the total rotations. Rats were given at least 15 minutes to acclimatize to the equipment after which the Roto Rat software was activated to record 30 minutes baseline prior to drug treatment. At 30 minutes the recording software was paused, rats were dosed with drug and placed back into the bowls and recording activity was re-started. The duration of the experiments ranges from 4-8 hours or until rotations have ceased for at least 1 hour. All timings for drugs administration unless stated otherwise in the figure legend will follow this timing regimen. Animals were assessed at 30-minute intervals for abnormal behavior. The number of rotations was then plotted as counts per 10 minutes. The area under the curve was

also calculated and this was plotted as total rotations per assessment period.

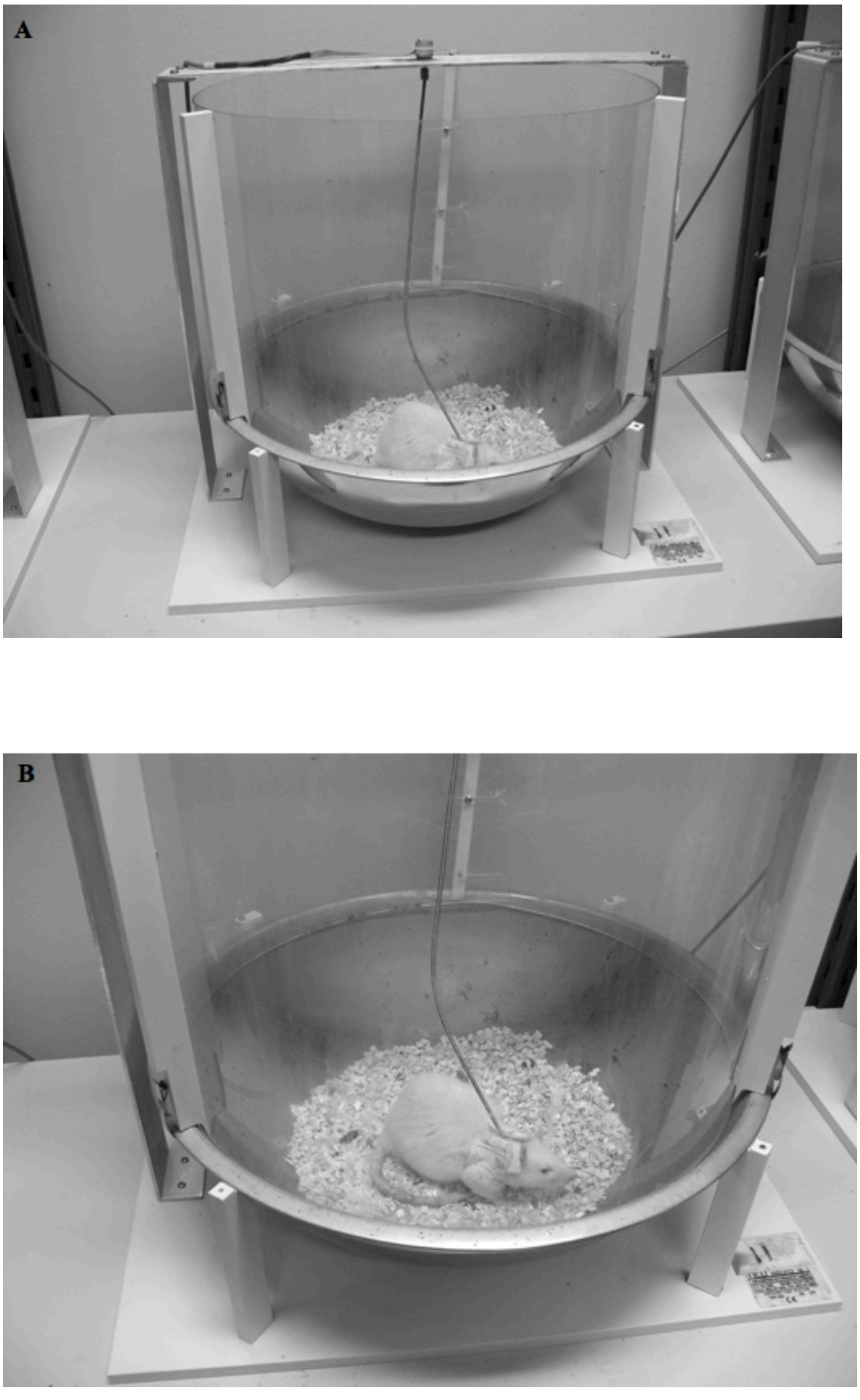


Figure 2. 4 A&B Rotometer test equipment

6-OHDA lesioned rat in placed in the harness and the tether is connected to the recording equipment.

2.3 The MPTP treated common marmoset

In humans, inadvertent systemic injection of the pethidine analogue 1-methyl–4-phenyl–1,2,3,6- tetrahydropyridine (MPTP) was found to rapidly induce a syndrome clinically indistinguishable from idiopathic Parkinson's disease. This was first reported in a single case study by Davis and colleagues (Davis et al. 1979) but was highlighted when 'synthetic heroin' containing the contaminant was injected by four Californian heroin addicts in the early 1980s, who rapidly developed a marked and sustained parkinsonism (Langston et al. 1983). This serendipitous discovery led to the development of the MPTP-treated non-human primate model of Parkinson's disease, which remains the most predictive of response in man, widely used (Jenner et al. 1984).

2.3.1 Neuropathological effects of systemic MPTP administration

Human and non-human primates are especially susceptible to MPTP, whereas rodents are relatively resistant. This interspecies variation is thought to be due, at least in part, to the high capacity of vesicular sequestration of MPTP that rodents exhibit (Sundstrom et al. 1986; Sundstrom et al. 1987) . In primates, MPTP induces a uniform loss of dopamine in both the caudate and putamen (Di Monte et al. 2000; Nomoto et al. 2000) unlike Parkinson's disease, where degeneration is more pronounced in the putamen (Kish et al. 1988). In contrast to Parkinson's disease patients, MPTP administration induces a rapid and non-progressive loss in dopaminergic neurons as well as the notable absence of Lewy

bodies. The MPTP- treated primate model therefore involves selective nigrostriatal neurodegeneration, but does not fully reflect all the characteristic pathologies of Parkinson's disease.

2.3.2 Behavioural effects of MPTP-induced lesion

MPTP toxicity induces the cardinal motor features of Parkinson's disease in both man and non-human primates, i.e. rigidity, akinesia and postural instability (Burns et al. 1983; Bloem et al. 1990). Resting tremor is seen in humans but rarely in primates (Langston et al. 1983; Bergman et al. 1998; Jenner 2003) although action tremor can be seen. MPTP-induced motor disability in primates is also responsive to anti-parkinsonian therapies prescribed in the clinic, including L-DOPA and dopamine agonists (Pearce et al. 1998) . The primary reasons for the MPTP model being of such value to the research community is the reproducibility of motor disability and dyskinesia, which allows new pharmacological therapies to be tested. Indeed, dyskinesia in primates is assessed using scoring criteria similar to those used clinically (Brotchie et al. 1999). Thus, the MPTP-treated common marmoset (Callithrix jacchus), as utilised in these studies, is one of the most commonly employed animal models today (Jenner 2009).

To date there is no more reliable an animal model of Parkinson's disease than the MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride) treated primate (Langston 1990; Eslamboli 2005). The neurotoxin MPTP was given systemically to common marmosets (2mg/kg,

s.c. for 5 consecutive days), which creates a reproducible model of Parkinson's disease. MPTP treated common marmosets display some of the cardinal signs of Parkinson's disease which include bradykinesia, rigidity and postural abnormalities, however only action tremor is observed (Langston 1985; Jenner 2003).

These motor deficits have been shown to be reversed by dopamine replacement therapy in the MPTP treated common marmoset using dopamine agonists or L-DOPA (Jenner 2003; Jenner 2004; Emborg 2007). The advantage of the MPTP non-human primate model is the ability to highlight the phenomenon of 'priming' for L-DOPA induced abnormal involuntary movements (dyskinesia). Using this animal model we can manipulate treatment combinations and patterns that could contribute to dyskinesia induction, priming, severity and or elimination.

2.3.3 Animals – MPTP treated common marmoset

Male and female adult common marmosets (350g or above) (source: Harlan UK) upon arrival at the site of designation were given at least two weeks to acclimatize to their new surroundings, cages and other marmosets prior to any treatments. During this period all animals were assessed for general health and body weights monitored to ensure they conform to the weight requirements on the project license (animals of 320g and above have a greater chance of surviving the acute effects of MPTP). Animals were housed alone or in pairs and were placed in holding rooms

which operate on a 12 hour light / dark cycle, 50% humidity at a temperature of 25 ± 1 ^{o}C. Animals had ad libutum access to Mazuri pellets and water. In addition they received two meals each day. In the morning they received mashed up Mazuri pellets, forage mix and pumpkin seeds. In the afternoon they received fresh fruit. All procedures are carried out in accordance with Home Office regulations under the Animals (Scientific Procedures) Act 1986 and with the approval of the Ethical Review Panel of King's College London. The project licence under which the MPTP marmoset work is carried out was 70/6345 or 70/7146.

2.3.4 MPTP administration

Locomotor and behavioral deficits were induced by subcutaneous administration of MPTP (2.0 mg/kg, s.c.; Sigma Chemical, UK) in sterile 0.9% saline solution (Baxter Healthcare Ltd.), daily for up to 5 consecutive days. Animals were then given 8-12 weeks to recover. By day three of MPTP administration animals become unable to feed or take care of themselves. From this point animals were hand fed everyday until the animals regained the ability to feed and groom themselves independently. Animals were weighed everyday from day one of MPTP administration and were given water and a high protein diet consisting of marmoset jelly, tamarin cake, blended bananas, whey protein powder shakes and *'complan'* food supplements (Jackson et al. 2004). During this recovery period animals developed a 'persistent climbing syndrome' whereby they climb in the home cages and rub there heads into the corners of the cage leading to

large skin abrasions. To prevent this, recovery units (fabric cage linings) have been designed which fit into the cages and prevent injury.

Weights were monitored closely to stipulate with Home Office requirements, which state that animals should not drop below 20% of their starting body weight.

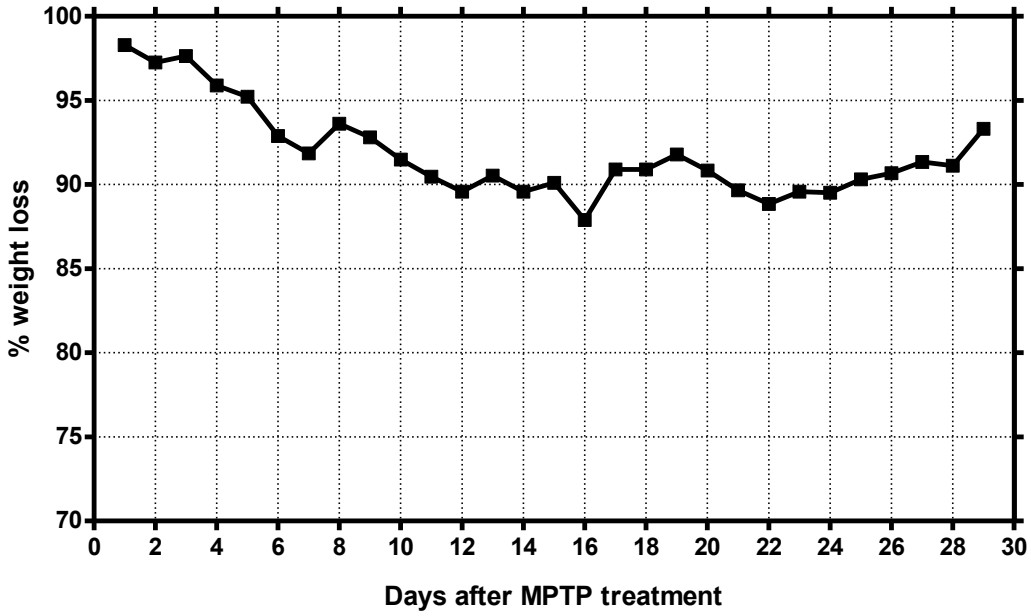


Figure 2. 5 Timeline of % weight change for MPTP marmosets after receiving MPTP

Common marmosets had received 5 days of MPTP (2mg/kg, SC) by day 0. Animals were weighed everyday for the first 30 days.

During this period MPTP marmosets were hand fed and weighed everyday to ensure body weight does not drop below 20%. N=4 MPTP treated common marmosets.

2.3.5 Induction of dyskinesia

When animals exhibit stable motor deficits including a marked reduction of basal locomotor activity, poor coordination of movement, abnormal and/or rigid posture, reduced alertness and head checking movements but can feed and groom themselves they were considered ready to begin L-DOPA treatment. Animals were primed to exhibit dyskinesia by the daily administration of L-DOPA (12.5 mg/kg, *'bis in die'* [B.I.D], p.o.) plus carbidopa (12.5mg/kg, B.I.D, p.o.) for up to 28 days. During this period animals progressively developed more severe dyskinesia, which plateaus and stabilizes (figure 2.6). Once primed, subsequent administration of a dopaminergic agent elicited the same dyskinetic response (Pearce et al. 1995; Emborg 2007). The effect of administered L-DOPA therapy was that motor disability is reversed, locomotor activity increases and a dyskinetic response is invoked (figure 2.11).

2.3.6 Behavioural assessment of the MPTP treated common marmoset

The assessment of the MPTP treated common marmosets consists of examining and scoring locomotor activity, motor disability reversal and dyskinesia. Dyskinesia is examined in 3 parts although only the overall dyskinesia is shown. Dyskinesia is categorized as chorea, dystonia and overall dyskinesia.

2.3.7 Locomotor activity in MPTP treated common marmoset

Animals were removed from the home cage and weighed. They were then transferred for acclimatization to the automated activity (test) units for 60 minutes during which a baseline locomotor activity was determined according to established protocols (Smith et al. 2003). Following drug administration locomotor activity was monitored for up to 10 hours. Each test unit was fitted with 8 photoelectric switches arranged to detect floor, perch and climbing activity (figure 2.7). Interruption of a photoelectric switch (infra red beam measuring activity at 8Hz) being automatically recorded as a single locomotor count. Locomotor counts were then accumulated and plotted as total counts per 30 minutes over the course of the test days to produce a time course of drug activity. From the time course data the area under the curve (AUC) was calculated and represents total locomotor activity over the duration of the experiment. All statistical analysis is carried out on the total AUC. The typical effect of L-DOPA in MPTP treated marmosets is shown in figure 2.7

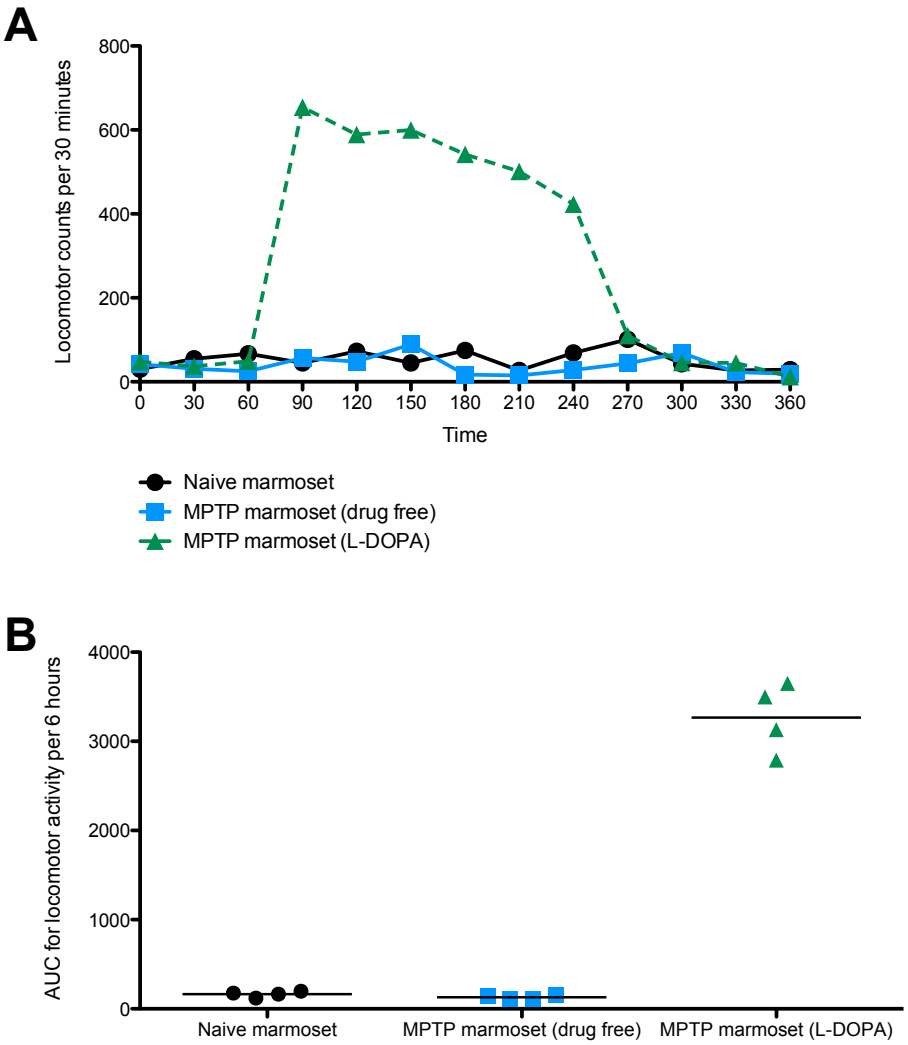


Figure 2. 6 Comparison of naive marmoset, MPTP treated marmoset and L-DOPA treated MPTP treated common marmoset locomotor activity

MPTP treated marmosets (n=4) were placed into test cages at t= 0 minutes and baseline locomotor activity, motor disability and dyskinesia were recorded for 60 minutes then at 60 minutes animals either received no treatment or L-DOPA (12.5mg/kg, p.o.) with carbidopa (12.5mg/kg, p.o.) and were observed in total for 6 hours. Data are expressed as time course (A) for locomotor activity and area under the curve (AUC) (B) as individual values for animals with the median value indicated.

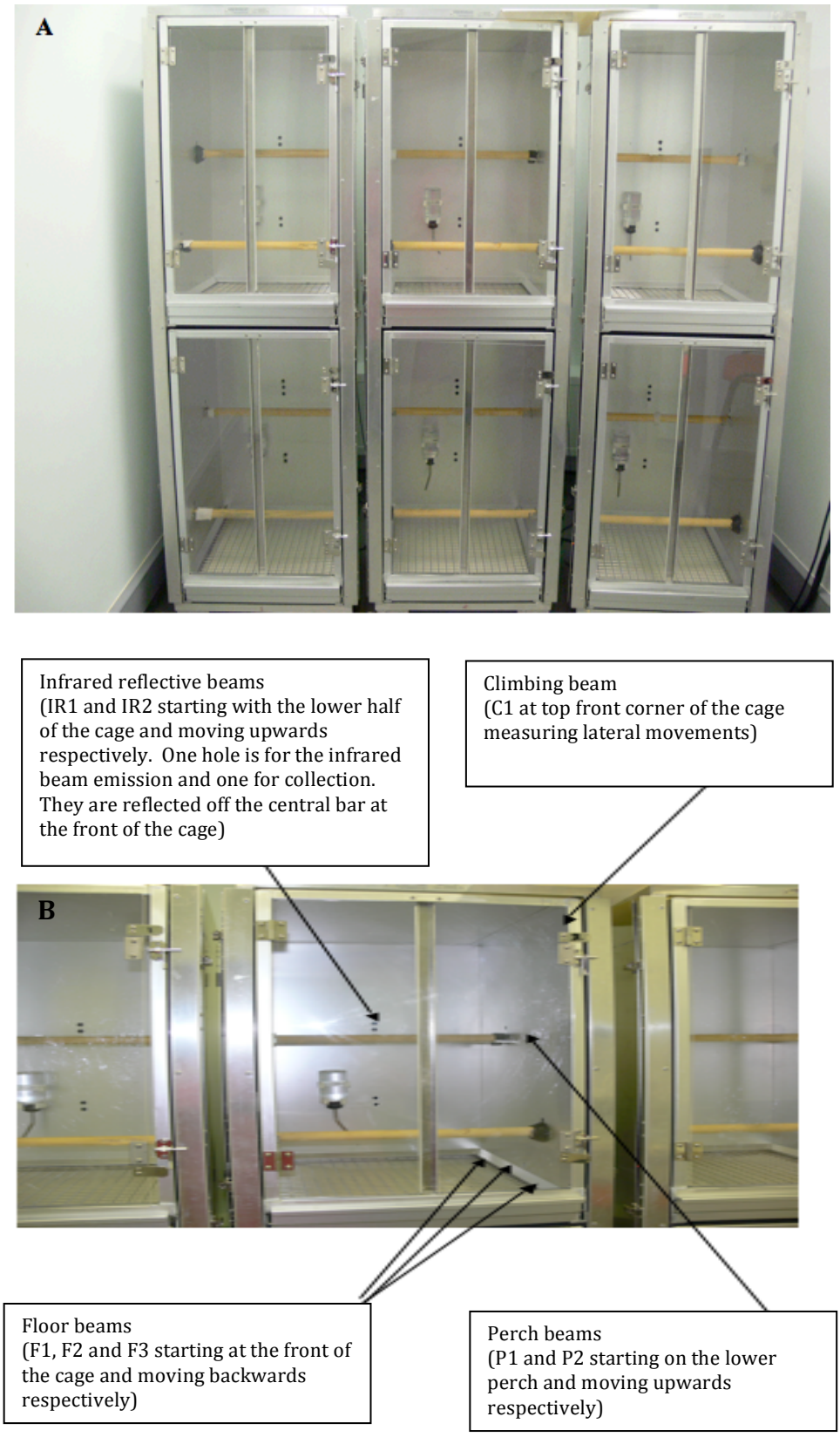


Figure 2. 7 Marmoset test units and placement of photoelectric beams

(A) shows the test cages as layed out in the room and (B) shows the position of the beams within the test cages which record movement

2.3.8 Motor disability in MPTP treated common marmoset

Motor disability was assessed as scheduled for locomotor activity. Assessment took place in the activity units at time = 50 to 60 minutes (basal assessment) and for the last 10 minutes of each 30 minute period (for example 20 to 30 minutes, 50 to 60 minutes) after drug administration for up to 8 hours. The assessment of motor disability was performed by an experienced observer using the following rating scale:

Motor Disability scoring scale					
Alertness	Normal (0)	Reduced (1)	Sleepy (2)		
Checking movements	Normal (0)	Reduced (1)	Absent (2)		
Posture	Normal (0)	Abnormal trunk (1)	Limbs (2)	Tail (3)	Grossly abnormal (4)
Balance	Normal (0)	Impaired (1)	Unstable (2)	Spontaneous falls (3)	
Reaction	Normal (0)	Reduced (1)	Slow (2)	Absent (3)	
Vocalization	Normal (0)	Reduced (1)	Absent (2)		
Motility	Normal (0)	Bradykinesia / reduced (1)	Akinesia / absent (2)		

The sum of the total scores was regarded as the motor disability score of each individual animal. Notes on other behaviours including stereotypy's,

vomiting, wet dog shakes and unusual behaviour such as: scratching, tracking (for example head tracking a non apparent moving object), explorative behaviour (includes foraging), staring at non apparent stimuli (increased focusing) and repetitive movements (circling cage floor with jumps and head checks in the same direction) were also noted. These additional observation were recorded but not used or plotted but could help determine overall tolerability of the drug as described with the use of the 5-HT1a agonist 8-OHDPAT (Iravani et al. 2006). Typical data showing the effect of L-DOPA on motor disability score are shown in figure 2.9.

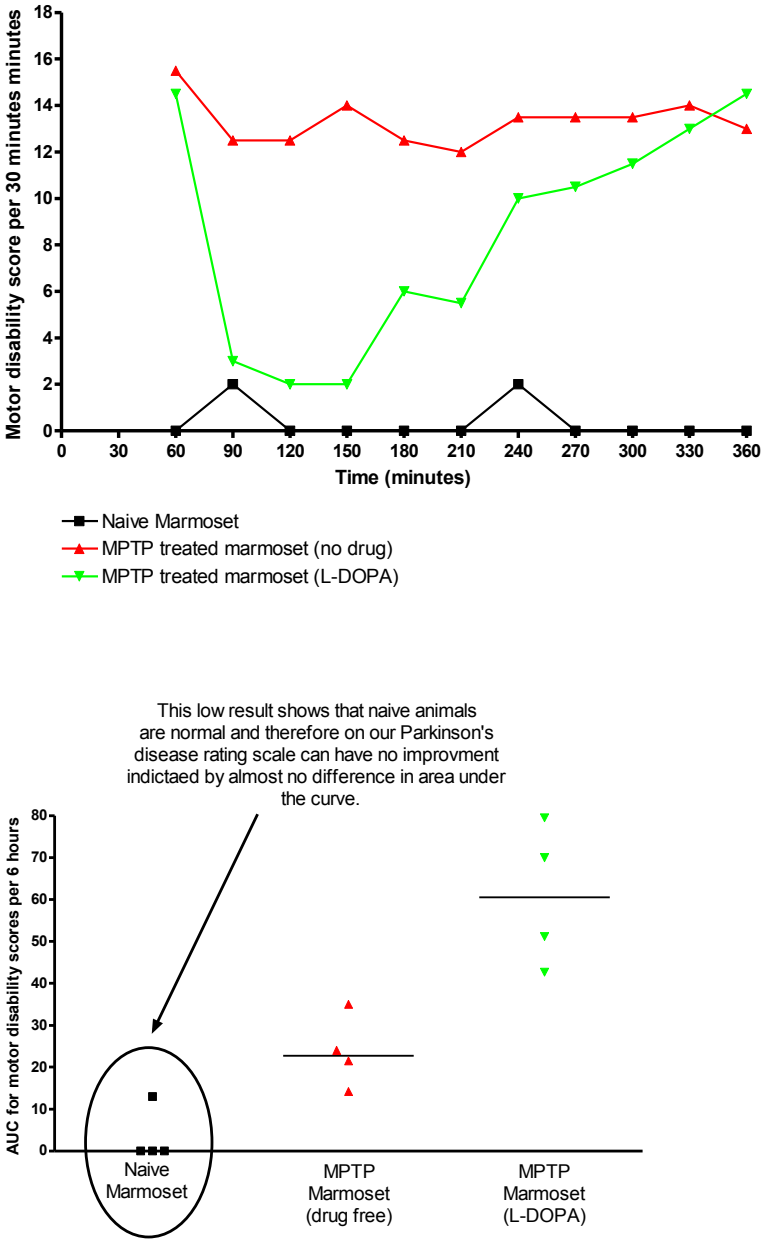


Figure 2. 8 Comparison of naive marmoset, MPTP treated common marmoset and L-DOPA treated MPTP treated common marmoset on motor disability

MPTP treated or naive marmosets (n=4) were placed into test cages at t= 0 minutes and baseline locomotor activity, motor disability and dyskinesia were recorded for 60 minutes then at 60 minutes animals either received placebo (sucrose water) or L-DOPA (12.5mg/kg, p.o.) with carbidopa (12.5mg/kg, p.o.) and were observed in total for 6 hours. Data are expressed as time course (A) for motor disability and totals area under the curve (AUC) (B) as individual values for animals with the median value indicated.

2.3.9 Dyskinesia in MPTP treated common marmoset

Dyskinesia was assessed as scheduled for locomotor activity. Assessment took place in the activity units at time = 50 to 60 minutes (basal assessment) and for the last 10 minutes of each 30 minute period (e.g. 20 to 30 minutes, 50 to 60 minutes) after drug administration for up to 8 hours. The assessment of dyskinesia was performed by an experienced observer who was blinded to the treatment regimen using the following rating scale:

Dyskinesia scoring scale	
0	Absent
1 (mild)	Fleeting and rare dyskinetic postures and movements
2 (moderate)	More prominent abnormal movements but not interfering significantly with normal behavior
3 (marked)	Frequent and at times continuous dyskinesia intruding upon normal repertory of activity
4 (severe)	Virtually continuous dyskinetic activity, disabling to animal and replacing normal behavior

This scale applies to both chorea and dystonia and the overall highest score in the observation period will be considered the score of the time (for example: chorea score of 4 with a dystonia score of 1 means that overall dyskinesia will be 4).

Definition of dyskinesia

Dystonia (arm, leg and trunk):

Abnormal sustained posture (e.g., leg elevation).

Athetosis (arm and leg):

Writhing twisting movements.

Chorea (arm and leg):

Abnormal rapid (dance like) movements of limbs.

Akathisia:

Motor restlessness.

Stereotypic reaching (arm):

Repeated, purposeless reaching movements

Typical data on the effect of L-DOPA on dyskinesia score is given in figure 2.10.

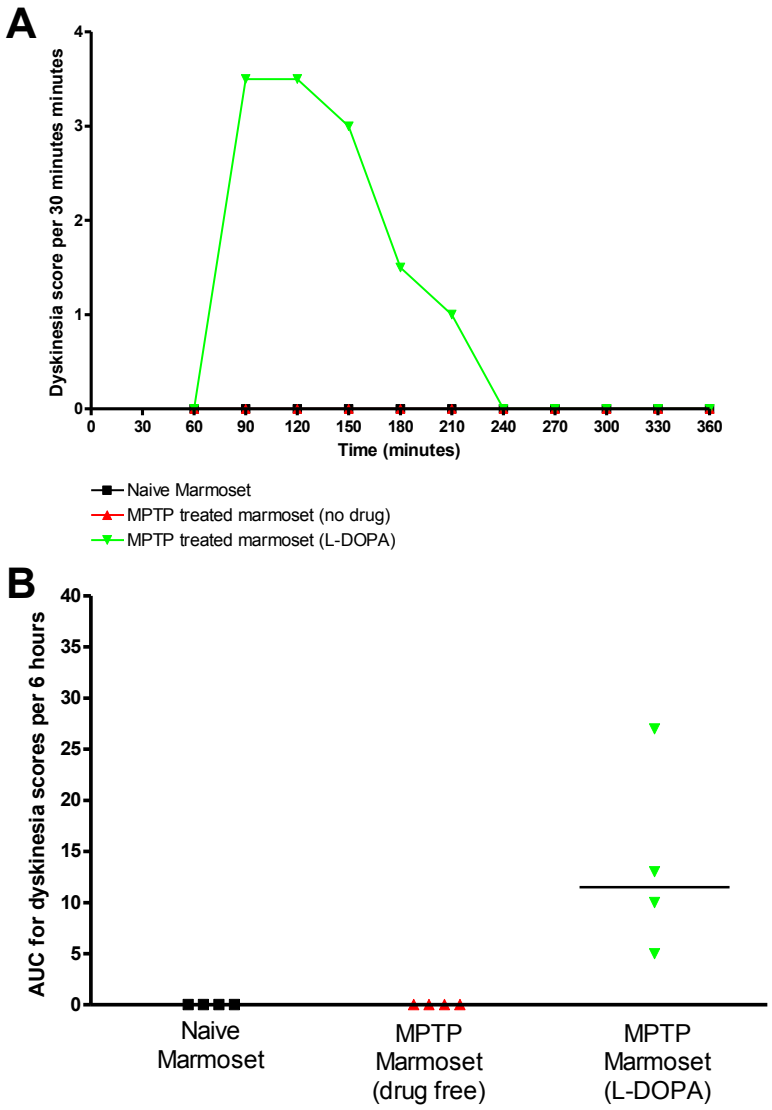


Figure 2. 9 Comparison of naive marmoset, MPTP treated marmoset and L-DOPA treated marmosets on dyskinesia

MPTP treated or naive marmosets (n=4) were placed into test cages at t= 0 minutes and baseline locomotor activity, motor disability and dyskinesia were recorded for 60 minutes then at 60 minutes animals either received sucrose water or L-DOPA (12.5mg/kg, p.o.) with carbidopa (12.5mg/kg, p.o.) and were observed in total for 6 hours. Data are expressed as time course (A) for dyskinesia and totals area under the curve (AUC) (B) as individual values for animals with the median value indicated.
Note: - the MPTP marmoset group given L-DOPA had received prior L-DOPA treatment and hence would be classed as primed animals.

All three of these behavioural parameters are combined on graphs similar to that below to produce an overall behavioural response to any particular therapy. It is the combined value of these 3 parameters, which determines the potential clinical value of a test drug.

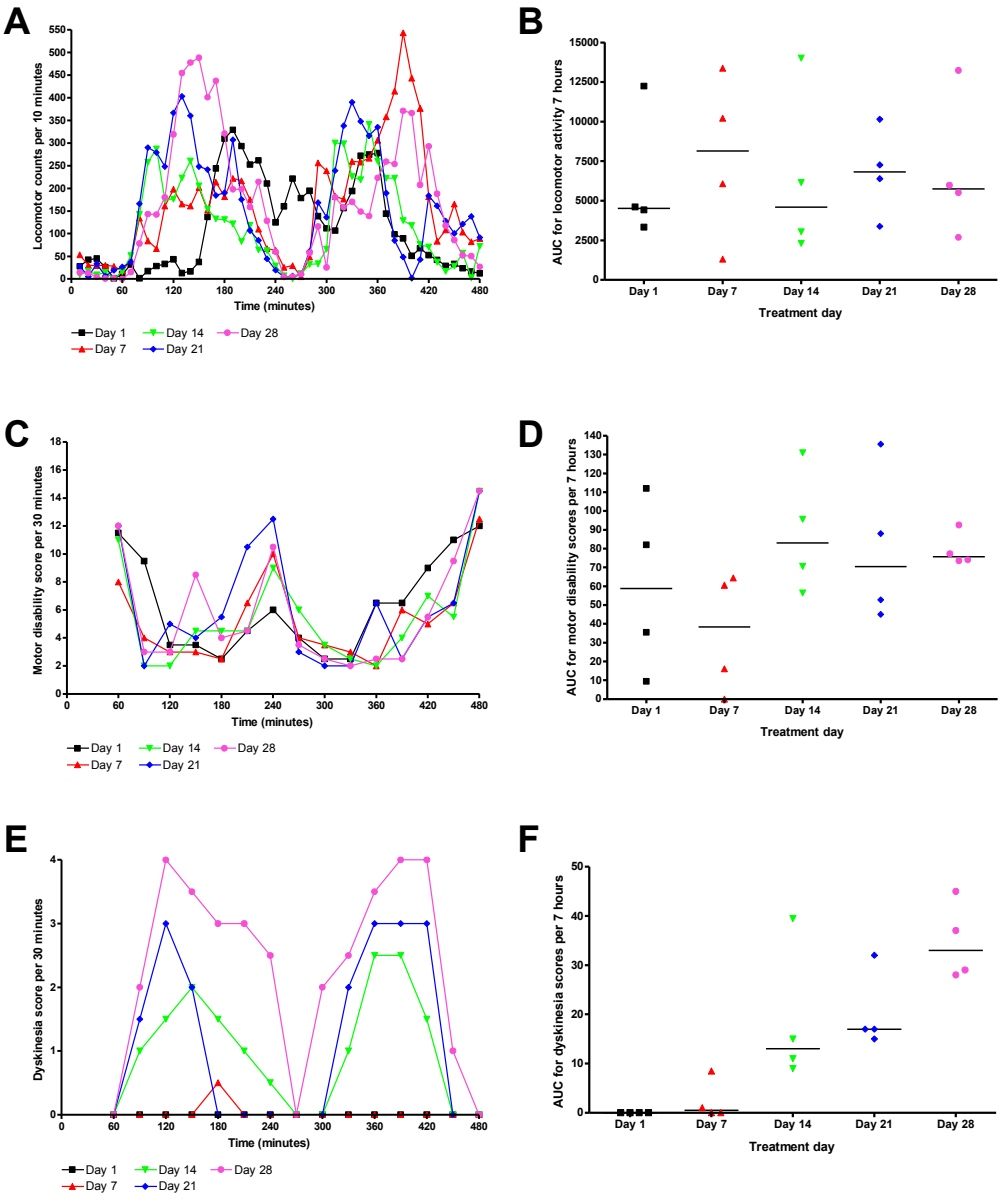


Figure 2.11 The effect of L-DOPA priming in de novo MPTP-treated common marmosets on locomotor activity, motor disability and dyskinesia induction

MPTP treated marmosets (n=4) were placed into test cages at t= 0 minutes and baseline locomotor activity, motor disability and dyskinesia were recorded for 60 minutes then at 60 and 270 minutes animals were treated with L-DOPA (12.5mg/kg, p.o.) with carbidopa (12.5mg/kg, p.o.) and were observed in total for 8 hours. Data are expressed as time courses (A, C and E) for locomotor activity, motor disability and dyskinesia respectively shown as median values with error bars omitted for clarity. Totals areas under the curve (AUC) are shown (B, D and F) as individual values for animals with the median value indicated.

An example of an overall marmoset score sheet for motor disability and dyskinesia is shown below as an example:

| OBSERVER RATING | | |

2.4 Data Evaluation of behaviour

Data was processed using Microsoft Excel, and GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com.

2.4.1 Analysis of 6-OHDA lesioned rat data

Roto Rat software version 2 records data into an Excel sheet as individual minute recordings of each individual animal that has been recorded. The software records partial rotations, which are sensitive to 45^{o} rotations. This means that one complete rat rotation was recorded as 8 counts (i.e. 360^{o} ÷ 45^{o} = 8). Data was recorded into two columns, which display counter clockwise rotations and clockwise rotations. Divide the value in these columns to show actual complete rotations both clockwise and counter clockwise per minute per animal. Subtract counter clockwise rotations from clockwise rotations and this calculates the total number of contralateral rotations per minute per animal as shown below:

Experiment:		DEMO			
Group:	1				
Box #: 6	Rat A				
Time Slice:	60 seconds				
Counts/Rev:	r) 45.00				
Retrace:					
Data (a/r):	File name				
	CW	CC	CW / 8	CC / 8	CW - CC
=========	========	==========			
Time Slice	Partial	Partial			
60	3	1	0.375	0.125	0.25
60	13	3	1.625	0.375	1.25
60	46	7	5.75	0.875	4.875
60	71	14	8.875	1.75	7.125
60	89	19	11.125	2.375	8.75
60	81	12	10.125	1.5	8.625
60	54	6	6.75	0.75	6
60	75	10	9.375	1.25	8.125
60	36	8	4.5	1	3.5
60	70	13	8.75	1.625	7.125
60	75	16	9.375	2	7.375
60	73	9	9.125	1.125	8

The individual number of turns per minute per animal was then collated into 10-minute time bins and these were used to plot a time course in GraphPad Prism version 4.00. AUC was calculated for each animal using the baseline or acclimatization period (for example: first 30 minutes animals are in the bowls without drug treatment which is typically ~ 0). This gave an overall value for total rotations for the duration of the experiment and this was then plotted for each treatment group of animals and these were then compared statistically between various treatment regimens or groups.

6-OHDA lesioned rat 'on-time' (defined as the amount of time that drug efficacy is inducing rotational behaviour) is calculated by combining the total number of minutes which 6-OHDA lesioned rats are rotating more than 2 turns per minute for more than 5 consecutive minutes.

2.4.2 Analysis of MPTP treated marmoset data

Locomotor activity data was collected in totals for 10 minute and 30 minutes time segments from individual animals. The 10-minute data was used to calculate the locomotor activity "on-time", where locomotor counts greater than 100 / 10 minutes were considered as an increase in activity and presented as the number of hours of "on-time". The 30-minute time segments were used to determine the area under the locomotor activity curve (AUC) for individual animals. Peak locomotor activity counts for individual animals were also determined. Motor disability was assessed for

10 minutes every 30 minutes for individual animals. The duration of motor disability reversal was indicated by scores less than 8 and presented as the number of hours of “on-time”. The 30-minute scores were used to determine the area under the motor disability curve (AUC) using the baseline score and the final score to define the baseline, and measuring the reduction from baseline as the AUC for individual animals. Maximum reversal of motor disability (minimum motor disability score) for individual animals was also determined. Dyskinesia was assessed for 10 minutes every 30 minutes for individual animals. The duration of troublesome (marked to severe) dyskinesia was indicated by scores greater than 2 and presented as the number of hours present. The 30-minute scores were used to determine the area under the dyskinesia curve (AUC) for individual animals. Peak dyskinesia for individual animals was also determined.

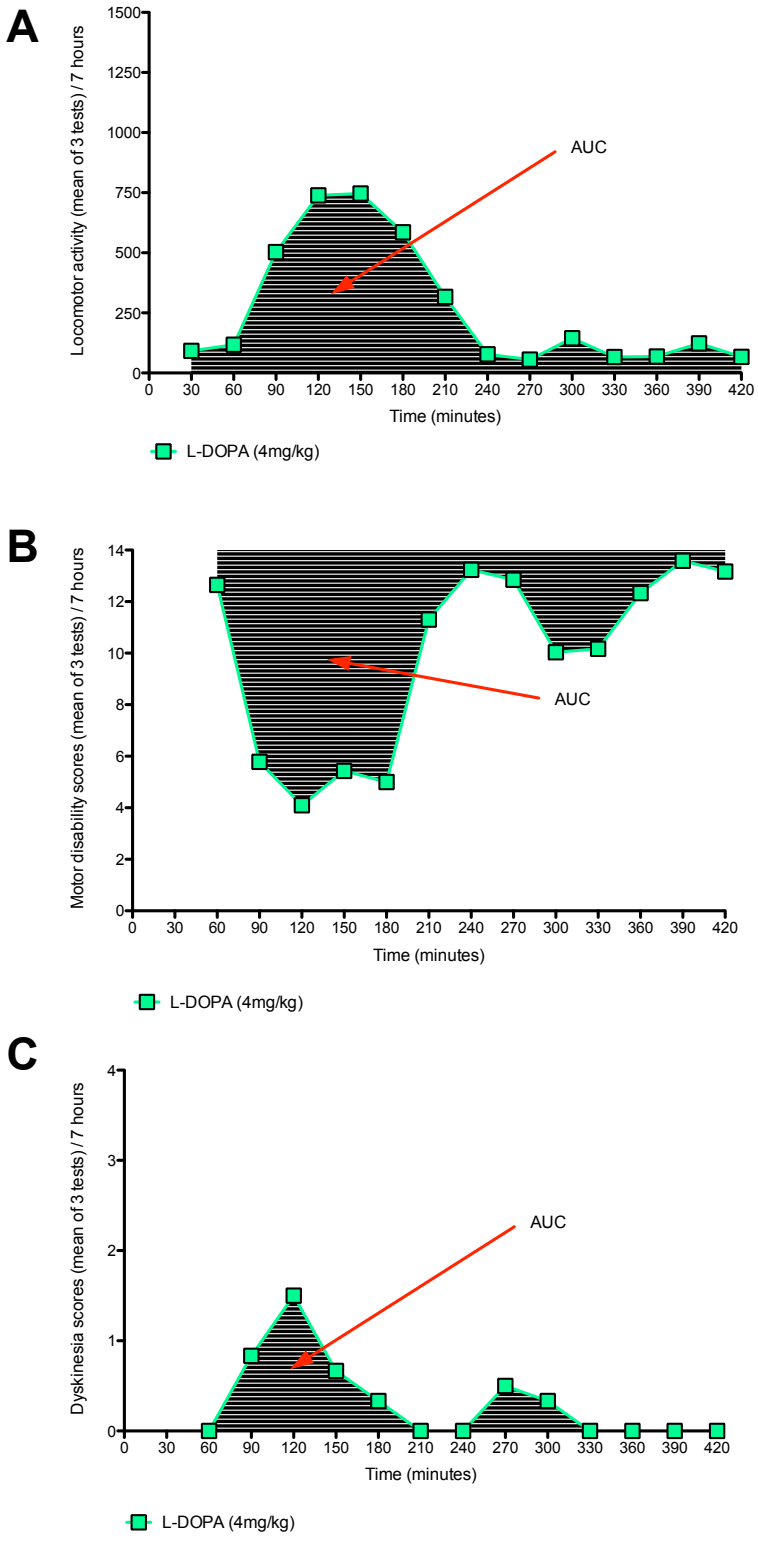


Figure 2.12 Definition of AUC, for locomotor activity, motor disability and dyskinesia. Figure A, B and C showing area under the calculations for locomotor activity, motor disability reversal and dyskinesia respectively.

Chapter 3

Benserazide dosing regimen affects the response to L-DOPA in the 6-OHDA lesioned-rat

3. Benserazide dosing regimen affects the response to L-DOPA in the 6-OHDA lesioned -rat

3.1 Introduction

The peripherally acting aromatic amino acid decarboxylase inhibitors (AADCIs), carbidopa and benserazide, are routinely used in the treatment of Parkinson's disease (Parkinson's disease) to inhibit the conversion of L-3,4-dihydroxyphenylalanine (L-DOPA) to dopamine in peripheral tissues. They potentiate the central action of L -DOPA on motor symptoms and reduce peripheral side-effects, such as nausea and hypotension (Ngwuluka 2010; Ngwuluka et al. 2010).

Current practice is that AADCIs are administered simultaneously in combined, fixed-ratio, formulations with L -DOPA, namely Sinemet (L-DOPA plus carbidopa) and Madopar (L -DOPA plus benserazide). This treatment regimen is used to maintain inhibition of peripheral AADC over the duration of the L -DOPA effect. However, the ratio of AADCIs to L-DOPA dose and the relative timing of administration appear untested. Indeed, it is not clear in which tissues AADC activity is inhibited and what the relative contribution of enzyme inhibition is to the overall L-DOPA effect. Similarly, it is unclear whether the effects of carbidopa and benserazide are the same and for how long inhibition is maintained after each dose.

Previous studies comparing carbidopa and benserazide in small numbers of patients found that they produced similar effects on the efficacy of L-

DOPA treatment (Greenacre et al. 1976). A more recent report, presented at the European Federation of Neurological Societies meeting in 2008 by Kuoppamäki et al., also indicates that combinations of L-DOPA plus either carbidopa or benserazide produce similar results (Kuoppamaki et al. 2009).

Although some information suggests that carbidopa is effective when given as a pretreatment to L -DOPA and that subsequent carbidopa treatment alone can further prolong the L -DOPA response (Bartholini and Pletscher. 1975), similar information with regard to benserazide is not available, despite its widespread use. The limited pharmacokinetic data reported suggests a half-life of 1–3 h for both drugs, suggesting that the inhibitory activity of DDCIs may be of relatively short duration (Da et al. 1987) but could potentially be as long as L-DOPA.

Thus, although DDCIs are routinely used in the treatment of Parkinson's disease, it is not clear whether the standard preparations are optimal for the enhancement of L-DOPA effects.

Benserazide is also routinely used to potentiate the effects of L -DOPA in animal models of Parkinson's disease, notably the 6-hydroxydopamine (6-OHDA) lesioned rat. A range of doses (5–20 mg/kg) and a variety of treatment times relative to the administration of L -DOPA are reported (Carey et al. 1994; Arai et al. 2003; Shen et al. 2003; Stefanova et al. 2004; Dekundy et al. 2007). However, there has been no systematic investigation in animal models of the relationship between dose and timing of administration, and duration of benserazide effect on the efficacy of L -

DOPA.

Thus it was hypothesised that L-DOPA induced contralateral rotations in the 6-OHDA lesioned-rat can be improved by optimising the dose and timing of the dopa-decarboxylase inhibitor.

3.2 Aims

In order to test this hypothesis, the studies described in this chapter aimed to assess the optimal conditions for AADC inhibition in order to achieve maximal enhancement of the effects of L-DOPA. The studies undertaken were:

a) To determine the effect of acclimatisation and time of day on L-DOPA induced contralateral rotations in the 6-OHDA lesioned rat
b) To establish the optimal pre-treatment dosing regimen with the AADCI benserazide on L-DOPA induced contralateral rotation in the 6-OHDA lesioned rat
c) To determine whether supplementary administration of benserazide potentiates L-DOPA induced rotation in the 6-OHDA lesioned rat

3.3 Methods and materials

The studies described in this chapter, aimed to establish the optimal dosing regime for benserazide on L-DOPA induced rotations in 6-OHDA lesioned rats. A brief overview of materials and methods used in this study are presented here but detailed protocols are to be found in Chapter 2 of the General Methodology.

3.3.1 Animals

Male Wistar rats weighing between 250-280g (source: Harlan UK) were given 1-week acclimatisation after arrival before surgery. Animals were kept on a 12 hour light / dark cycle (7am – 7pm) at approximately 50% humidity and room temperature of ~20°C. Rats had access to Purina rat food pellets and water ad libutum when in the home cage. For details of 6-OHDA lesions refer to section 2.2.5. Following surgery, animals were challenged with L-DOPA plus benserazide to prime for peak and stable rotational behaviour measured as described in section 2.2.7. On test days, animals were given up to 60 minutes acclimatisation prior to drug treatment unless stated otherwise.

3.3.2 Drugs

All drugs were made up in 0.9% saline and given orally by gavage in a volume of 1ml/kg.

3.3.3 Surgery and animal selection

Following 6-OHDA lesion (described in section 2.2.5), male Wistar rats were given two weeks for the lesion to stabilise and for the acute effects of general anaesthesia to have worn off. Animals at this point were acutely challenged with apomorphine (0.5mg/kg, s.c.) to confirm lesion authenticity (figure 2.2). Animals displaying greater than 5 turns per minute at peak activity (~ 30 minutes post injection) were selected for future studies. This is a proven and approved method of determining a lesion of the MFB (medial forebrain bundle) of greater than 95% (Paxinos et al. 1985).

3.4 Benserazide dose response study in 6-OHDA lesioned rats in combination with standard L-DOPA dose

6-OHDA lesioned rats (n=7) were removed from home cage, body weight recorded and then placed into rotometers attached to the hanging tether. Rats were given up to 30 minutes to acclimatise and then the recording software started. Baseline values for individual rats were recorded for 30 minutes (-30mins to 0mins). Animals were then dosed with benserazide (doses utilised were 0, 3.125, 6.25, 10 and 15mg/kg) on separate days in combination with L-DOPA (12.5mg/kg, p.o.) and left in the bowls. The testing period post drug administration lasted for 300 minutes and total recording time was 330 minutes.

Net contralateral rotations were plotted as time course graph and from this data total Area Under the Curve (AUC) was calculated as detailed in section

2.4.1. Animals were given at least 3 days washout prior to next drug treatment. The administration of drug treatment was carried out as a Latin square (rats were randomly assigned a treatment selection depending on test day).

	Time course of activity	
	-30 to 0 mins	60 - 300 mins
Group 1	Baseline	L-DOPA (12.5mg/kg) + vehicle p.o.
Group 2	Baseline	L-DOPA + benserazide (3.125mg/kg) p.o.
Group 3	Baseline	L-DOPA + benserazide (6.25mg/kg) p.o.
Group 4	Baseline	L-DOPA + benserazide (10mg/kg) p.o.
Group 5	Baseline	L-DOPA + benserazide (15mg/kg) p.o.

3.5 The effect of acclimatisation on the rotational response of 6-OHDA lesioned rats prior to L-DOPA with benserazide administration

6-OHDA lesioned rats (n=8) were removed from home cage, body weight recorded and then placed into rotometers attached to the hanging tether. Rats were given up to 30 minutes to acclimatise and then the recording software started. Baseline values for individual rats were recorded for 30 minutes (-30mins to 0mins). Animals were then left in the bowls with the recording software running. Then at either 60, 120 or 180 minutes and at each of these time points on separate days animals were dosed with L-DOPA (12.5mg/kg, p.o.) with benserazide (10mg/kg, p.o.). Doses of benserazide and L-DOPA used were based on previous studies carried out by our group as well being reported in the literature. The dose of benserazide used was 12.5mg/kg, which is considered a high dose in order

to ensure high levels of peripheral DDC inhibition, with reports in the literature being dose ranges from 3.25mg/kg through to 25mg/kg (Lane et al. 2006; Marin et al. 2009). The testing period post drug administration lasted for 330 minutes and total recording time was 360 minutes.

Net contralateral rotations were plotted as time course graph and from this data total Area Under the Curve (AUC) was calculated as detailed in section 2.4.1. Animals were given at least 3 days washout prior to next drug treatment. The administration of drug treatment was carried out as a Latin square (rats were randomly assigned a treatment selection depending on test day).

	Time course of activity				
	-30 to 0 mins	60 mins	120 mins	180 mins	330 mins
Group 1	baseline	L-DOPA (12.5mg/kg) + benserazide (10mg/kg), p.o.			
Group 2	baseline		L-DOPA (12.5mg/kg) + benserazide (10mg/kg), p.o.		
Group 3	baseline			L-DOPA (12.5mg/kg) + benserazide (10mg/kg), p.o.	

3.6 The effect of different timings of dopa-decarboxylase inhibitor pre-treatment on the rotational response of 6-OHDA lesioned rats prior to L-DOPA administration

6-OHDA lesioned rats (n=4) were removed from home cage, body weight recorded and then placed into rotometers attached to the hanging tether. Rats were given up to 30 minutes to acclimatise and then the recording software started. Baseline values for individual rats were recorded for 30 minutes (-30mins to 0mins). At 0 minutes all animals were dosed with

benserazide (10mg/kg, p.o.) then at either 60, 120 or 180 minutes were dosed with L-DOPA (12.5mg/kg, p.o.). The testing period post drug administration lasted for 330 minutes and total recording time was 360 minutes.

Net contralateral rotations were plotted as time course graph and from this data total Area Under the Curve (AUC) was calculated as detailed in section 2.4.1. Animals were given at least 3 days washout prior to next drug treatment. The administration of drug treatment was carried out as a Latin square (rats were randomly assigned a treatment selection depending on test day).

	Time course of activity					
	-30 to 0 mins	0 mins	60 mins	120 mins	180 mins	330 mins
Group 1	baseline	benserazide (10mg/kg), p.o.	L-DOPA (12.5mg/kg), p.o.			
Group 2	baseline	benserazide (10mg/kg), p.o.		L-DOPA (12.5mg/kg), p.o.		
Group 3	baseline	benserazide (10mg/kg), p.o.			L-DOPA (12.5mg/kg), p.o.	

3.7 The effect of supplemental benserazide administration on the L-DOPA induced rotational response in 6-OHDA lesioned rats

6-OHDA lesioned rats (n=8) were removed from home cage, body weight recorded and then placed into rotometers attached to the hanging tether. Rats were given up to 30 minutes to acclimatise and then the recording software started. Baseline values for individual rats were recorded for 30 minutes (-30mins to 0mins). At 0 minutes all animals were dosed with L-

DOPA (12.5mg/kg, p.o.) with benserazide (10mg/kg, p.o.). Then at 120 minutes, animals were either given no treatment, saline or an additional dose of benserazide (10mg/kg, p.o.). The testing period post initial drug administration lasted for 270 minutes and total recording time was 300 minutes.

Net contralateral rotations were plotted as time course graph and from this data total Area Under the Curve (AUC) was calculated as detailed in section 2.4.1. Animals were given at least 3 days washout prior to next drug treatment. The administration of drug treatment was carried out as a Latin square (rats were randomly assigned a treatment selection depending on test day).

	Time course of activity			
	-30 to 0 mins	0 mins	120 mins	270 mins
Group 1	baseline	L-DOPA (12.5mg/kg) + benserazide (10mg/kg), p.o.	Benserazide (10mg/kg) p.o.	
Group 2	baseline	L-DOPA (12.5mg/kg) + benserazide (10mg/kg), p.o.	Vehicle p.o.	
Group 3	baseline	L-DOPA (12.5mg/kg) + benserazide (10mg/kg), p.o.		

3.8 Results

3.8.0 Benserazide dose response study in 6-OHDA lesioned rats in combination with standard L-DOPA dose

L-DOPA (12.5mg/kg, p.o.) alone as expected produced a small and short rotational response (figure 3.0). With increasing doses of benserazide in combination with L-DOPA, the rotational response in the 6-OHDA lesioned rats increased and became significantly different to L-DOPA alone at both 10mg/kg and 15mg/kg.

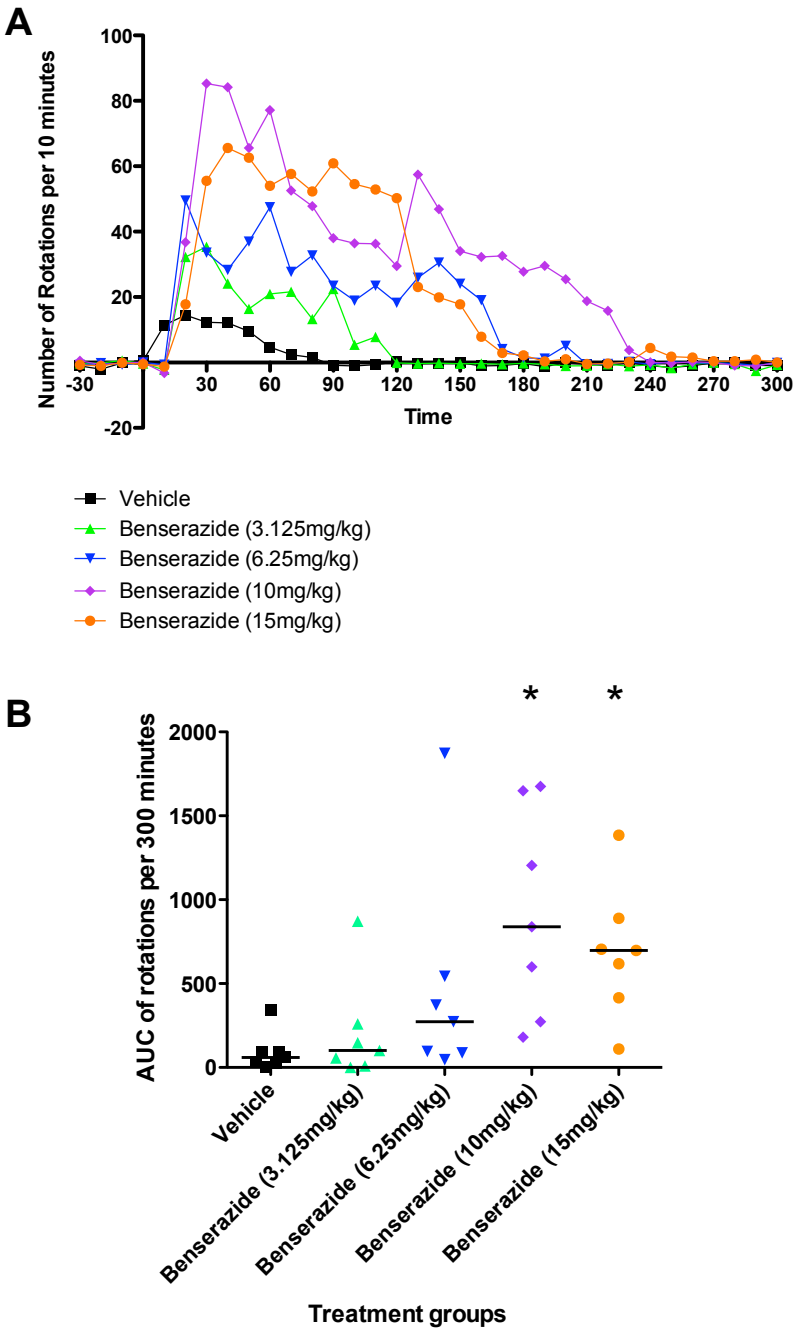


Figure 3.0 Dose response study for benserazide in 6-OHDA lesioned rats

Rotational response of 6-OHDA lesioned rats (n=7) after administration of L-DOPA (12.5mg/kg, p.o.) concomitantly with benserazide (0, 3.125, 6.25, 10 and 15mg/kg, p.o.) at 0 minutes. Time course data expressed as median (A) and total area under the curve expressed as individual values with the median indicated (B). Administration of L-DOPA and benserazide (10 and 15mg/kg) produced a significantly increased rotational response compared to L-DOPA alone. * $P < 0.05$ compared to L-DOPA (12.5mg/kg, p.o.) alone. Repeated measures ANOVA followed by Dunnett's post hoc test on transformed data, $y=\sqrt{y}$.

3.8.1 The effect of acclimatisation on the rotational response of 6-OHDA lesioned rats prior to L-DOPA with benserazide administration

L-DOPA (12.5mg/kg, p.o.) with benserazide (10mg/kg, p.o.) induced a contralateral rotational response in the 6-OHDA lesioned rats when given after 60, 120 or 180 minutes acclimatisation periods (figure 3.1A). Rotational activity reduced such that the longer the rats were left to acclimatise in the rotometers, the lower the response (figure 3.1B). Although the greatest number of contralateral rotations were produced by administration of L-DOPA and benserazide after 60 minutes of acclimatisation, this was not significantly different to L-DOPA and benserazide administration at 120 minutes. However, the number of rotations observed after L-DOPA and benserazide administered at 180 minutes was significantly reduced compared to those after L-DOPA and benserazide administered at 60 minutes (figure 3.1B). L-DOPA and benserazide administered at 60, 120 and 180 minutes produced rat 'on-times' of 166 minutes, 89 minutes and 93 minutes. Three out of the 8 animals that were dosed with L-DOPA and benserazide at 120 minutes or at 180 minutes did not respond to the treatment.

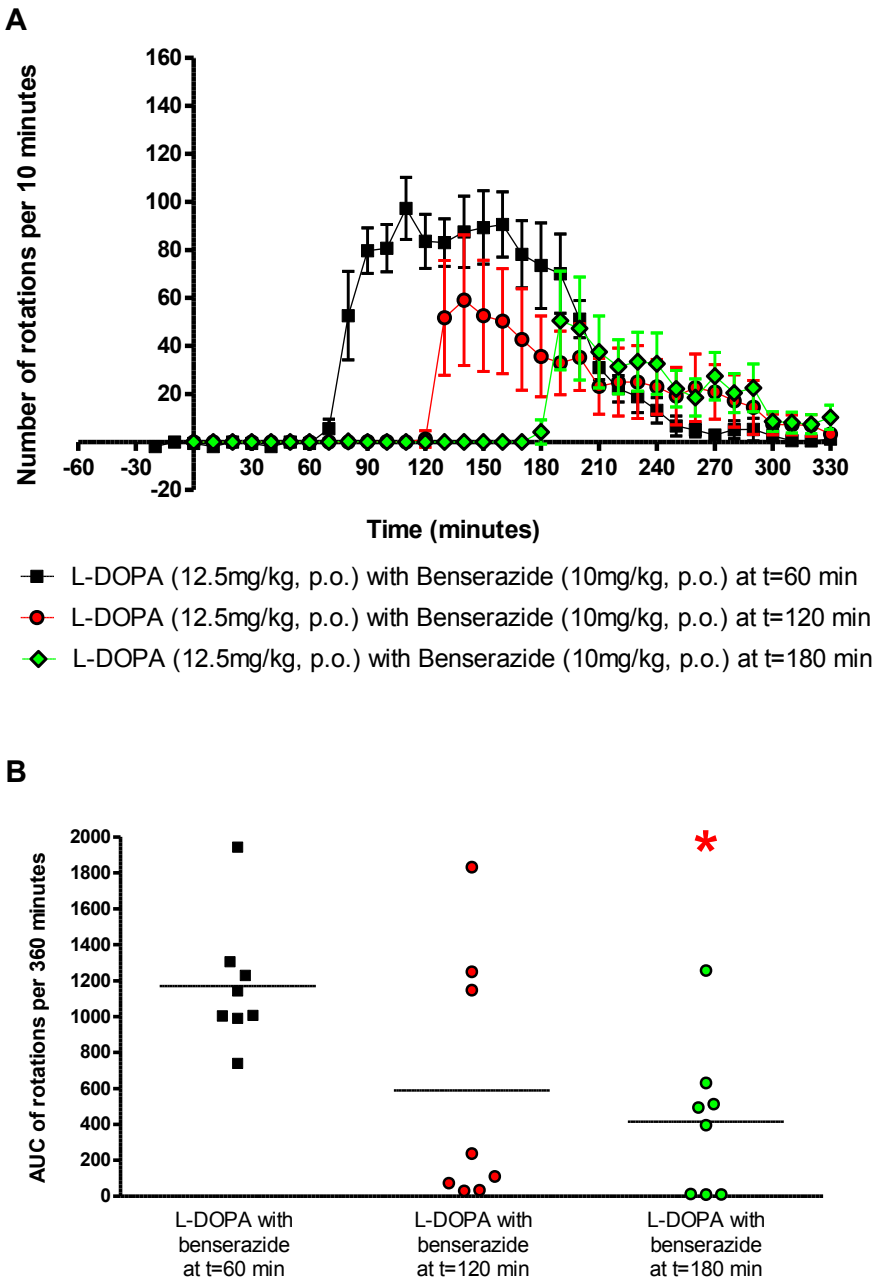


Figure 3. 1 The effect of acclimatization on the rotational response of 6-OHDA lesioned rats prior to administration of L-DOPA and benserazide

Rotational response of 6-OHDA lesioned rats (n=8) after administration of L-DOPA (12.5mg/kg, p.o.) concomitantly with benserazide (10mg/kg, p.o.) at 60, 120 and 180 minutes after acclimatization. Time course data expressed as mean ± SEM (A) and total area under the curve expressed as individual animals with the mean value for the group (B). Administration of L-DOPA and benserazide at 180 minutes produced a significantly reduced rotational response compared to L-DOPA and benserazide dosed at 60 minutes. * $P < 0.05$ compared to L-DOPA (12.5mg/kg, p.o.) with benserazide (10mg/kg, p.o.) dosed at 60 minutes, repeated measures ANOVA followed by Dunnett's post hoc test on transformed data, $y=\sqrt{y}$.

3.8.2 The effect of different timings of benserazide pre-treatment on the rotational response of the 6-OHDA lesioned rats to L-DOPA with benserazide administration

There was no significant difference in the number of L-DOPA (12.5mg/kg, p.o.) induced rotations produced by pre-treating animals with benserazide (10mg/kg, p.o.) at increasing time intervals of 60, 120 and 180 minutes prior to L-DOPA (figure 3.2A and 3.2B). Similarly, the duration of response measured as 'on-time' was not significantly different for the 3 treatment regimes being 100, 73 and 55 minutes respectively. The measurement of 'on' time is calculated as the duration of time which rats rotated greater than 2 turns per minutes for more than 5 consecutive minutes.

Whilst it may initially appear that concomitant treatment produces a higher AUC compared to L-DOPA with L-DOPA following a 60-minute pre-treatment with benserazide, it should be noted that these were carried out in different animals. The groups themselves are acting as a control between the experiments assessing pre-treatment and those assessing acclimatisation.

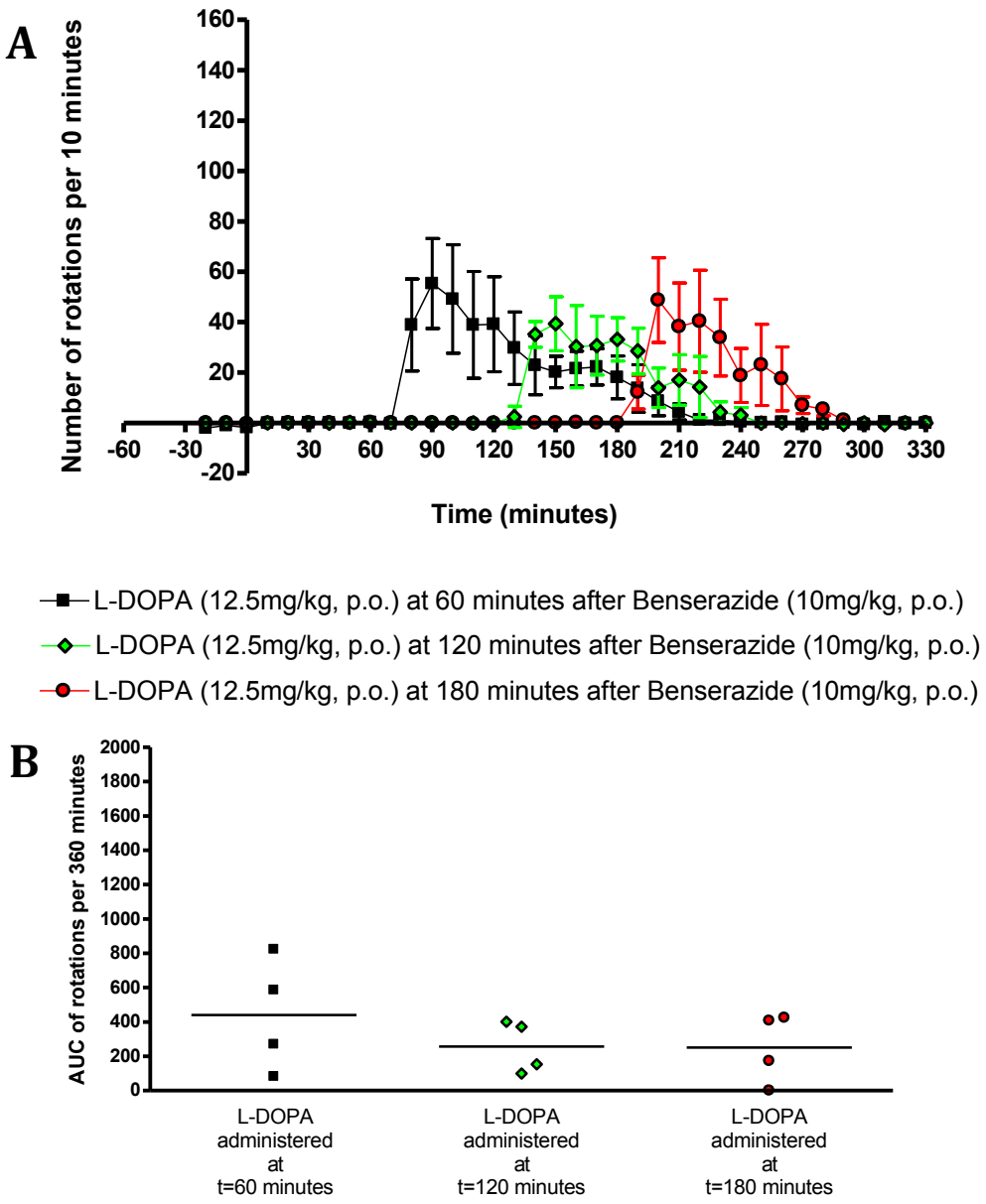


Figure 3. 2 The effect of different timings of dopa decarboxylase inhibitor pre-treatment on the rotational response of the 6-OHDA lesioned rats to L-DOPA

Rotational response of 6-OHDA lesioned rats (n=4) after administration of L-DOPA (12.5mg/kg, p.o.) at t=60, 120 or 180 minutes following pre-treatment with benserazide (10mg/kg, p.o.) at t=0 minutes. Time course data expressed as mean ± SEM (A) and total area under the curve expressed as individual animals with the mean value for the group (B). Increasing the duration of pre-treatment between benserazide and L-DOPA administration had no significant effect on contralateral rotations up to 180 minutes. $P > 0.05$ compared to L-DOPA (12.5mg/kg, p.o.) dosed at t=60 minutes after benserazide (10mg/kg, p.o.), repeated measures ANOVA followed by Dunnett's post hoc test on transformed data, $y=\sqrt{y}$.

3.8.3 The effect of supplemental benserazide administration on the L-DOPA induced rotational response in 6-OHDA lesioned rats

As expected, L-DOPA plus benserazide increased rotational behaviour for up to 120 minutes (figure 3.3A and 3.3C). At 120 minutes following the administration of L-DOPA plus benserazide, the administration of an additional dose of benserazide (10mg/kg p.o.) resulted in a significant increase in the peak of contralateral rotations compared to the administration of vehicle at this time point (figure 3.3B). This resulted in a significant difference in AUC between 120-270 minutes (figure 3.3B). Whilst not carried out in these experiments, it would be assumed that due to the rapid metabolism of L-DOPA in the periphery, the initial administration of L-DOPA alone followed by a subsequent DDCI dose one or more hours later would not have had the same effect. Majority of L-DOPA would be rapidly metabolised by DDC and COMT.

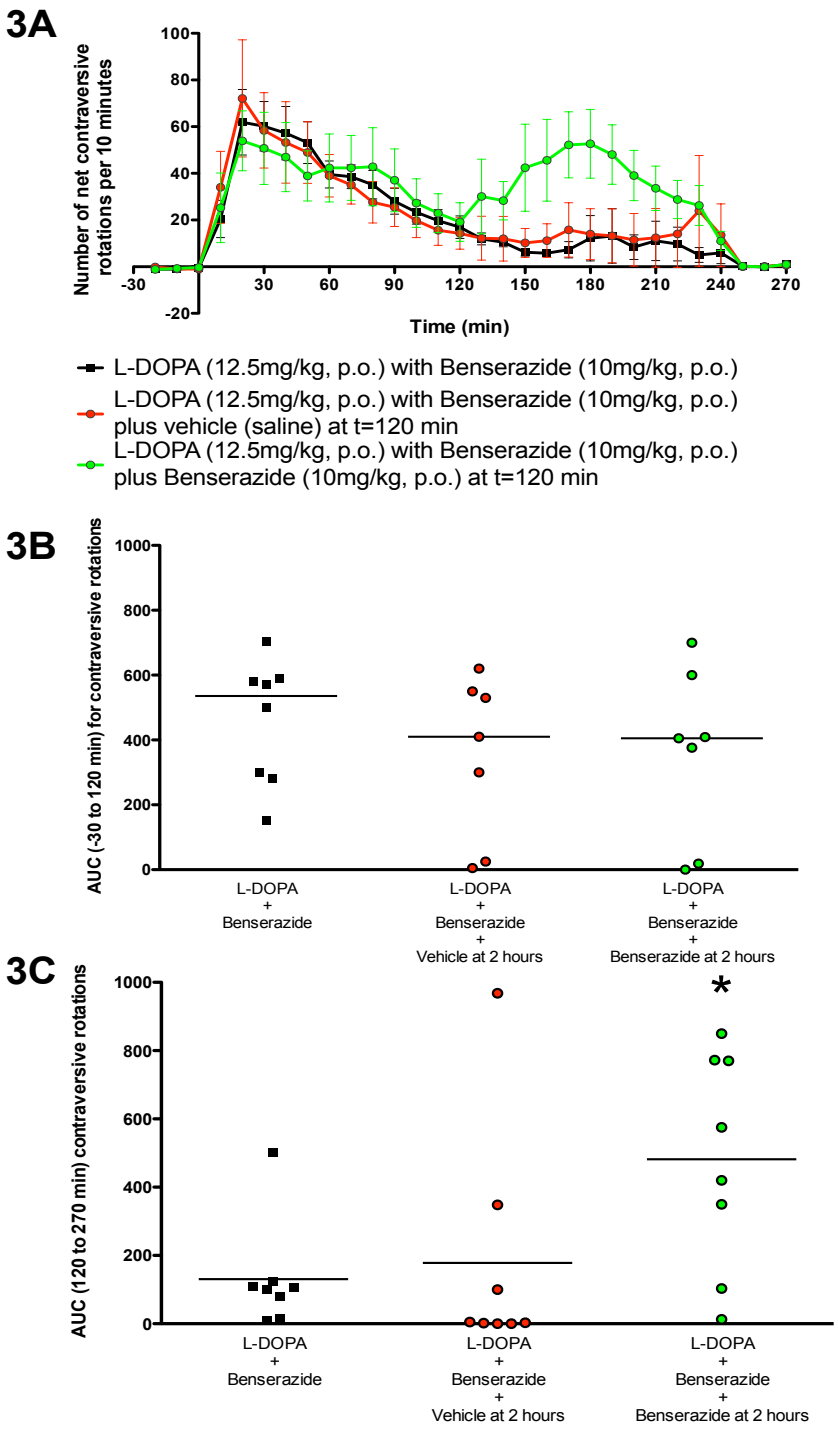


Figure 3. 3 The effect of supplemental benserazide on L-DOPA induced contralateral rotations in the 6-OHDA lesioned rats Rotational response of 6-OHDA lesioned rats after administration of L-DOPA (12.5mg/kg, p.o.) concomitantly with benserazide (10mg/kg, p.o.) and with either no treatment, saline or supplemental benserazide (10mg/kg, p.o.) 120 minutes. (A) Time course (mean ± SEM), (B) Total area under the curve (mean ± SEM) for the first 120 minutes and (C) for the period 120 – 270 minutes. * Indicates L-DOPA plus supplemental benserazide is significantly different to L-DOPA (12.5mg/kg, p.o.) without supplemental benserazide, P = 0.0028, F = 9.173, One Way ANOVA followed by Dunnett's *post hoc* test.

3.9 Discussion

L-DOPA remains the most effective drug for the treatment of Parkinson's disease, since the discovery of its ability to reverse motor deficits exactly 50 years ago (Hornykiewicz 2001). In the ensuing 50 years, numerous studies have looked at the pharmacokinetic and pharmacodynamic profiles of L-DOPA (Nutt et al. 1984; Nutt et al. 1986; Nutt 2008) but in contrast, there have been very few investigations of the factors affecting the actions of AADCIs and this may have limited the most effective management of Parkinson's disease with L-DOPA. Even in experimental models of Parkinson's disease, little has been done to optimize the efficacy of dosage and treatment regimens. Thus it was hypothesised that L-DOPA induced contralateral rotations in the 6-OHDA lesioned-rat can be improved by optimising the dose and/ or timing of the dopa-decarboxylase inhibitor. In order to test this hypothesis we investigated the effect of different doses of benserazide, which showed significant improvement with higher doses of benserazide, acclimatisation and time of day on L-DOPA induced contralateral rotations in the 6-OHDA lesioned rats. We demonstrated that rotational responses could be significantly reduced with increased acclimatisation periods. We also investigated the optimal pre-treatment dosing regimen with the DDCI, benserazide on L-DOPA induced contralateral rotation in the 6-OHDA lesioned rat, which showed no significant overall effect on rotational response. Lastly we demonstrated that supplementary administration of benserazide potentiates L-DOPA induced rotations in the 6-OHDA lesioned-rat.

This study is the first to report on the effect of varying the dosage and timing of the AADCI benserazide with L-DOPA in 6-OHDA-lesioned rats, despite this model having been in common use since the mid-1960s (Holtz 1959; Ungerstedt 1968). Oral administration was utilised for all treatments of both drugs to reflect clinical use, although parenteral administration is commonly used in experimental models of Parkinson's disease (Treseder et al. 2000; Picconi et al. 2008). For this reason, benserazide, which is more soluble, was utilised over carbidopa in this series of experiments. Whilst there is no conclusive evidence of whether oral treatment is different to parenteral treatment in the 6-OHDA lesioned rat, we know from routine experience that differences are limited to the onset of rotations being more rapid to start but no other noticeable effects on duration or peak rotations. The results showed, both from a dose and a time perspective, how benserazide treatment influences the ability of L-DOPA to induce contralateral rotation.

It is common practice in the 6-OHDA-lesioned rat to administer AADCIs with L-DOPA and was used as the starting point. Benserazide was combined with a dose of L-DOPA known to be effective in inducing a substantial rotational response after decarboxylase inhibition (Gerlach et al. 2013; Da Prada et al. 1987). As expected, L-DOPA alone produced no significant behavioural response, but when combined with benserazide in doses up to 10 mg/kg (p.o.), there was a dose-related increase in the peak rotational response and in the duration of the effect on behaviour. Indeed, 10 mg/kg of benserazide was probably optimal, as at a higher dose there

was not only no further improvement in response, but a slight, non-significant reduction in both the peak and duration of rotational response. The reasons for this require further investigation but the most likely explanation is that at this dose level benserazide starts to inhibit central decarboxylase activity and this then inhibits the conversion of L-DOPA to dopamine. Indeed, early studies that established the fixed ratios of AADCIs to L-DOPA found a similar effect (Hodge et al. 1964; Bartholini et al. 1975). More recent rat studies measuring dopamine levels and AADC activity using micro-dialysis in both the intact and the denervated striatum, indicate that inhibition of central decarboxylase activity does occur with increased doses of benserazide (Jonkers et al. 2001; Shen et al. 2003). These findings show very clearly that the dosage level of AADCIs used in the 6-OHDA lesioned rats is a critical factor in behavioural outcome. This may even vary between rat strains (Eskow Jaunarajs et al. 2010) and so needs to be optimised in individual laboratories.

The alternative strategy used in 6-OHDA-lesioned rats is to use pre-treatment with AADCIs, but as little is known about the pharmacokinetic profile of these drugs relative to L -DOPA, optimization is required. Early studies suggest that there is approximately 80–90% inhibition of AADC in rodents 3 h after benserazide administration, at least in the kidney and liver (Da et al. 1987), but how this relates to the extent of L -DOPA decarboxylation appears unknown. Therefore, we gave benserazide at the optimal dose of 10 mg/kg, 1, 2, and 3 h before L –DOPA treatment. Pre-treatment by even 1 h tended to reduce the rotational response observed

compared with simultaneous administration, suggesting that decarboxylase inhibition is established quickly but that the duration of effect of benserazide treatment is short and corresponds to the apparent duration of effect of L -DOPA (data not presented but the analysis was carried out).

At longer pre-treatment times, the response to L -DOPA administration declined further, suggesting that inhibition of decarboxylase activity was not maximally effective in ensuring L -DOPA delivery to the brain. This also suggested that in the rat, the half-life of benserazide in plasma (or the half-life of decarboxylase inhibition) is not that different from the apparent duration of action of L-DOPA and that at later time points, a loss of decarboxylase inhibition may occur that results in L-DOPA having a shorter duration of activity. This is supported by the supplemental benserazide dose (figure 3.3). This was tested by giving a second dose of benserazide, 2 h after the administration of simultaneous treatment with L -DOPA plus benserazide. The result was a re-emergence of rotational response, suggesting that indeed, a relatively short period of full inhibition of AADC occurs in acute benserazide treatment. All of this has implications for how benserazide is used in experiments in 6-OHDA-lesioned rats and it may well have implications for how treatment with benserazide should be applied in Parkinson's disease. In a clinical setting when L-DOPA doses are changed it is not clear whether clinicians take account of DDCI dose and as shown, too high a dose can reduce the optimal response (if we assume that rotational behaviour is reflective of clinical L-DOPA improvement) or too low a dose and the clinical meaningful L-DOPA dose is not experienced

(figure 3.0).

These studies were designed to investigate the effect that pre-treatment has on L-DOPA induced rotational activity, and the discussion so far appears perfectly reasonable and logical for the effects observed. We were taken by surprise by the results of giving concomitant administration of L-DOPA and benserazide at the same times of day as covered by the experiments on the effects of pre-treatment. However, it might be interpreted that that time of day exerts a significant effect on the L-DOPA response. There was little difference between the rotational responses seen after drug administration at 08.00h (0 mins) and 09.00h (60 mins) but the behavioural effects were markedly reduced when treatment was started at 10.00h (120 mins) or 11.00h (180 mins).

Because these effects were so unexpected, further time points were subsequently investigated, as the findings raise important questions. They also suggest that the effects of pre-treatment with benserazide may have a more complex explanation than at first glance. The lesser effect of L-DOPA could be due to a change in the peripheral or central pharmacokinetics of the drug, which would require measurement of plasma and brain profiles and rates of conversion to dopamine.

The most obvious factor affecting L-DOPA bioavailability would be its absorption, which can be erratic and affected by food consumption and gastric emptying. L-DOPA-induced contralateral rotations might also be affected by food intake, as dietary amino acids compete with L -DOPA for transport across the blood–brain barrier (Del Amo et al. 2008). However,

as feeding in rats is nocturnal (Terrón et al. 2013), there would be a greater risk of impaired absorption and brain penetration early in the day compared with later on in the day. The alternative explanation is that this is a pharmacodynamic effect due to diurnal variation in the firing of dopaminergic neurones, changes in receptor trafficking or daily patterns of behavioural repertoire. Indeed, circadian rhythm influences amphetamine-induced stereotypy in the normal rat although general locomotor activity is less affected (Gaytan et al. 1998). Perhaps, more importantly, 6-OHDA lesioning of the striatum disrupts circadian rhythms in heart rate, temperature, and activity, which can be restored by chronic L -DOPA treatment (Ben et al. 1999; Boulamery et al. 2010; Gravotta et al. 2011).

However, in contrast, the time of day when drug is administered has no effect on the expression of abnormal involuntary movement in the 6-OHDA-lesioned rat (Monville et al. 2009). Clearly, this is a complex area, and while it is easy to speculate, these initial findings suggest that far more detailed exploration of this possible chronobiological phenomenon is required. In conclusion, the results of this study illustrate a very simple point, that is the dose and timing of the administration of benserazide is key to the behavioural effects of L-DOPA observed in the 6-OHDA lesioned rat, but this is rarely controlled for in experiments utilising this model. However, unexpectedly, the investigations raise the intriguing issue of variation in the response to L–DOPA with time of day, which is an issue of importance for understanding the nature of dopaminergically mediated behaviours and for the treatment of Parkinson's disease.

Summary

The experiments in this chapter set out to test the hypothesis that L-DOPA induced contralateral rotations in the 6-OHDA lesioned-rat can be improved by optimising the dose and timing of the dopa-decarboxylase inhibitor. The results in this chapter show that benserazide dose is critical to optimal rotational behaviour in 6-OHDA lesioned rats with 10mg/kg showing the greatest L-DOPA induced response (figure 3.0). Dosing rats with L-DOPA and benserazide simultaneously later in the morning resulted in a drop in rotational behaviour (determined by AUC) that reached significant levels where as the effect of pre-treatment with benserazide prior to L-DOPA dose did not have a significant impact even up to three hours. The addition of a supplemental dose of benserazide showed significant increase in rotational behaviour compared to the timeline of a standard dose of L-DOPA and benserazide.

This chapter demonstrated that our hypothesis is true but the 6-OHDA lesioned rat is not a reliable predictor of the clinical response and therefore the work in this chapter has not allowed us to evaluate the effects on motor disability and dyskinesia. The following chapter will evaluate these responses in the MPTP-treated common marmoset, which produces responses more clinically relevant.

Chapter 4

The timing of administration, dose dependence and efficacy of dopa decarboxylase inhibitors on the reversal of motor disability produced by L-DOPA in the MPTP treated common marmoset

4. The timing of administration, dose dependence and efficacy of dopa decarboxylase inhibitors on the reversal of motor disability produced by L-DOPA in the MPTP treated common marmoset

4.1 Introduction

In the previous chapter, it was established that L-DOPA induced contralateral rotations in the 6-OHDA lesioned-rat can be improved by optimising the dose and timing of the dopa-decarboxylase inhibitor, benserazide. Whilst this is of interest to non-clinical researchers, it does not shed light on motor disability and dyskinesia complications, which are a major complication of disease progression and side effect profile of drug treatment. The non-human primate model (MPTP-treated common marmoset) acts as a close and reproducible model to mimic certain characteristics of drug treatment that appears in man. In this chapter, the work carried out in chapter 3 is advanced to reflect a more clinically meaningful model, assess motor disability and dyskinesia components of treatment and provide a more holistic overview of how the efficacy of L-DOPA can be improved by reducing the expression of dyskinesia whilst maintaining optimal motor function in Parkinson's disease.

Carbidopa and benserazide are typically administered simultaneously with each dose of L-DOPA. This presumes that the onset of decarboxylase

activity inhibition occurs sufficiently rapidly to prevent peripheral L-DOPA metabolism and that inhibition persists during the time course of effect of L-DOPA (Pinder et al. 1976). The plasma half lives (t1/2) of carbidopa and benserazide are approximately 2.5 h and this correlates with the extent of decarboxylase activity inhibition (Lieberman et al. 1975; Korten et al. 1975; Da et al. 1987).

However, carbidopa pre-treatment was shown to reduce the rate of intravenous L-DOPA infusion required to achieve therapeutic L-DOPA plasma levels compared to simultaneous carbidopa treatment (Nutt et al. 1985).

Similarly, in normal rats, carbidopa administered 30 min prior to L-DOPA (i.v. and i.p.) produced a significant increase in the AUC (area under the curve) and half-life for L-DOPA in plasma compared to simultaneous administration (Leppert et al. 1988). This suggests that prior inhibition of DDC is more effective for carbidopa. However, there are no similar studies on the most efficient timing of benserazide administration.

Clinical studies have not shown any significant difference in clinical benefit between the two dopa-decarboxylase inhibitors carbidopa and benserazide (Marsden et al. 1973; Admani et al. 1985). However, higher plasma levels of L-DOPA were achieved when administered in conjunction with benserazide compared to carbidopa, although the doses of dopa-decarboxylase inhibitors used may not have been equivalent (Hagan et al. 1980).

Primate models are widely used for the assessment of symptomatic treatments in Parkinson's disease. Their primary benefit over rodent models is their ability to reflect motor disability and dyskinesia so as to allow some clinical interpretations prior to drugs being given to man. In experimental models of Parkinson's disease there is little evidence of how dopa-decarboxylase inhibitors should be used in conjunction with L-DOPA. There have not been investigations of the timing and dosage of dopa-decarboxylase inhibitors in 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP)- treated primates. In fact, the doses and timings for dopa-decarboxylase inhibitor use employed in the MPTP-treated primate vary markedly between laboratories and largely appear historical in nature (Close et al. 1985; Quik et al. 2002; Fox et al. 2002; Iravani et al. 2006; Samadi et al. 2007; Campos-Romo 2008). In this model, carbidopa and benserazide doses range between 1 and 50 mg per kg with both concomitant and pre-treatment protocols being utilised. As a consequence, it is unknown whether these dosing regimens, result in an optimal potentiation of the effects of L-DOPA or not. Whilst on a clinical level, the decision of whether a patient is prescribed Sinemet or Madopar is determined on their geography and the relevant marketing authorization for the drugs available, to date no new DDCI's are being prescribed with L-DOPA. As discussed earlier (section 1.5.8), the chemical structure of carbidopa and benserazide are similar and as such a similar drug derivative of these compounds, L-AMD (L-alpha-methyl dopa) which is currently prescribed as Aldomet for hypertension (Mah et al. 2009) was also of interest to be studied in this thesis. The effects of L-AMD as a potential

dopa-decarboxylase inhibitor for use in Parkinson's disease (Hess 1961; Yitey-Smith et al. 1970) have not been investigated in MPTP treated common marmosets nor in the 6-OHDA lesioned rat. Whilst a considerable effort has been undertaken to find new drug treatment options, both benserazide and carbidopa have been unchallenged as part of the "L-DOPA gold standard" treatment regimen. Here for the 1st time we used the old drug, L-AMD, due to its similar chemical structure and reported DDCI properties (Mah et al. 2009) in novel ways to assess whether the choice of the DDCI is important in optimising L-DOPA induced behaviour particularly focused on motor disability reversal and dyskinesia expression and whether we may need to consider the choice of DDCI on a more clinical and patient centric perspective.

The studies described in this chapter set out to test the hypothesis that L-DOPA induced dyskinesia and reversal of motor disability can be improved by altering the dose, timing and choice of dopa-decarboxylase inhibitor used in combination with L-DOPA.

4.2 Aims

In order to test this hypothesis, these studies aimed to compare the two most commonly used dopa-decarboxylase inhibitors, benserazide and carbidopa, in the MPTP treated common marmoset on L-DOPA induced reversal of motor disability and expression of dyskinesia. In addition the potential use of L-AMD as a dopa-decarboxylase inhibitor was explored. Specifically these aimed:

a) To establish the dose response relationship of carbidopa and benserazide to L-dopa on motor function in MPTP treated common marmosets.
b) To establish the effect of acclimatisation on the motor response to L-DOPA and a dopa-decarboxylase inhibitor in MPTP treated common marmosets.
c) To establish the optimal time interval between DDCI administration and L-dopa treatment on motor function and dyskinesia.
d) To compare the effects of the non-clinically used DDCI for Parkinson's disease, L-AMD to carbidopa and benserazide on motor function and dyskinesia in the MPTP treated common marmoset.

4.3 Methods and materials

The studies described in this chapter, aimed to establish the optimum-dosing regimen of a DDCI with L-DOPA based on the behavioural response in MPTP-treated common marmosets in terms of locomotor activity, motor disability and dyskinesia. A brief overview is presented here but detailed protocols are to be found in the General Methodology, Chapter 2.

4.3.1 Animals

Male and female adult common marmosets (350g or above) (Harlan UK) were given two weeks to acclimatize prior to MPTP treatment. Animals were housed either in single cages or in male and female pairs providing the male was vesectomised. Animals were placed in holding rooms which operate on a 12 hour light / dark cycle, 50% humidity at a temperature of 25 ± 1. Animals had *ad libutum* access to Mazuri pellets and water at all times. For details of MPTP treatment please refer to section 2.3. Following MPTP treatment, animals were allowed 8-12 weeks for motor deficits to stabilize and then primed with L-DOPA plus carbidopa unless stated other wise in the figure legend. On test days, animals were given at least 60 minutes acclimatization in the test cage prior to drug treatment unless stated in the figure legend.

4.3.2 Drugs

All drugs were made up in 10% sucrose solution and administered by oral gavage in a volume of 2ml/kg.

4.3.3 Animal selection

Following MPTP administration (refer to section 2.3) animals were treated chronically with L-DOPA (12.5mg/kg, p.o.) and carbidopa (12.5mg/kg, p.o.) B.I.D for up to 28 days or until steady dyskinetic behaviour was observed on each future challenge with L-DOPA or dopamine agonist. Animals were assessed for 1) locomotor activity, 2) motor disability and 3) dyskinesia as described in section (section 2.3.6). Animals were then assigned into groups of 6 ensuring groups were balanced based on their locomotor activity (section 2.6A) activity between groups they have similar median locomotor counts. Animals demonstrating poor L-DOPA response (e.g. low locomotor activity, brief or no reversal of motor disability and little or no dyskinesia) were not used for future studies.

4.4 Investigation of the effect of dose of dopa-decarboxylase inhibitors on motor function

MPTP-treated common marmosets (n=6) were placed into behaviour cages on 9 separate occasions with at least 2 days wash out between test days for the same DDCI and 1 week between different DDCIs. Following a 60 minute baseline, animals were dosed with L-DOPA (12.5mg/kg, p.o.) plus either benserazide or carbidopa (0, 3.125, 6.25, 9.375 or 12.5mg/kg, p.o.). The experimental design was carried out as a Latin square. Behavioural assessment was then carried out as described in section 2.3.6 and statistical analysis was performed as described in section 2.4.2.

	Time course of activity		
	0 - 60 mins	60 mins	60 - 360 mins
Group 1	baseline	L-DOPA (12.5mg/kg) p.o.	
Group 2	baseline	L-DOPA (12.5mg/kg) + benserazide (3.125mg/kg), p.o.	
Group 3	baseline	L-DOPA (12.5mg/kg) + benserazide (6.25mg/kg), p.o.	
Group 4	baseline	L-DOPA (12.5mg/kg) + benserazide (9.375mg/kg), p.o.	
Group 5	baseline	L-DOPA (12.5mg/kg) + benserazide (12.5mg/kg), p.o.	
Group 6	baseline	L-DOPA (12.5mg/kg) + carbidopa (3.125mg/kg), p.o.	
Group 7	baseline	L-DOPA (12.5mg/kg) + carbidopa (6.25mg/kg), p.o.	
Group 8	baseline	L-DOPA (12.5mg/kg) + carbidopa (9.375mg/kg), p.o.	
Group 9	baseline	L-DOPA (12.5mg/kg) + carbidopa (12.5mg/kg), p.o.	

4.5 Investigation of the effect of acclimatisation on motor function induced by L-DOPA and benserazide administration in the MPTP treated marmoset

MPTP-treated common marmosets (n=6) were placed into behaviour cages on 3 separate occasions with at least 2 days wash out. Following a 60-minute baseline, for either 60, 120 or 180 minutes before dosing with L-DOPA (12.5mg/kg, p.o.) plus benserazide (10mg/kg, p.o.). The experimental design was carried out as a Latin square. Behavioural

assessment was then carried out as described in section 2.3.6 and statistical analysis was performed as described in section 2.4.2.

	Time course of activity				
	0 - 60 mins	60 mins	120 mins	180 mins	420 mins
Group 1	baseline	L-DOPA (12.5mg/kg) + benserazide (10mg/kg), p.o.			
Group 2	baseline		L-DOPA (12.5mg/kg) + benserazide (10mg/kg), p.o.		
Group 3	baseline			L-DOPA (12.5mg/kg) + benserazide (10mg/kg), p.o.	

4.6 Investigation of the effect of timing of dopa-decarboxylase inhibitor administration

MPTP-treated common marmosets (n=6) were dosed with benserazide (10mg/kg, p.o.) and placed into behaviour cages on 3 separate occasions with at least 2 days wash out. Animals were then at either 60, 120 or 180 minutes dosed with L-DOPA (12.5mg/kg, p.o.). The experimental design was carried out as a Latin square. Behavioural assessment was then carried out as described in section 2.3.6 and statistical analysis was performed as described in section 2.4.2.

	Time course of activity				
	0 mins	60 mins	120 mins	180 mins	420 mins
Group 1	benserazide (10mg/kg), p.o.and baseline	L-DOPA (12.5mg/kg) p.o.			
Group 2	benserazide (10mg/kg), p.o.and baseline		L-DOPA (12.5mg/kg) p.o.		
Group 3	benserazide (10mg/kg), p.o.and baseline			L-DOPA (12.5mg/kg) p.o.	

4.7 Investigation of the effects of L-AMD on L-DOPA induced motor function

MPTP-treated common marmosets (n=6) were placed into behaviour cages on 4 separate occasions with at least 2 days wash out. Animals were then at 60 minutes dosed with L-DOPA (12.5mg/kg, p.o.) plus either carbidopa, benserazide or L-AMD (12.5mg/kg, p.o.). The experimental design was carried out as a Latin square. Behavioural assessment was then carried out as described in section 2.3.6 and statistical analysis was performed as described in section 2.4.2.

	Time course of activity		
	0 mins	60 mins	60 - 360 mins
Group 1	Baseline	L-DOPA (12.5mg/kg) p.o.	
Group 2	Baseline	L-DOPA (12.5mg/kg) + carbidopa (12.5mg/kg) p.o.	
Group 3	Baseline	L-DOPA (12.5mg/kg) + benserazide (12.5mg/kg) p.o.	
Group 4	Baseline	L-DOPA (12.5mg/kg) + L-AMD (12.5mg/kg) p.o.	

4.8 Results

4.8.1 Investigation of the effect of dose of dopa decarboxylase inhibitors on motor function

Two dose response studies were carried out in the same MPTP treated common marmosets (n=6) using doses of 3.125, 6.25, 9.375 and 12.5mg/kg orally in combination with L-DOPA which would allow examination of the dose ratio of L-DOPA : DDCI to determine the effect on motor disability reversal and dyskinesia expression. The two DDCI's were examined separately first and are then compared to each other.

4.8.1.1 Carbidopa dose response study

The administration of L-DOPA (12.5mg/kg, p.o.) alone induced a small increase in locomotor activity, reversed motor disability to its maximum peak for a short time period (between 90-120min) and induced mild to moderate dyskinesia. Two of the six animals had a poor response whereby locomotor activity, motor disability and dyskinesia did not go above baseline values (figure 4.1A, C & E).

The administration of L-DOPA (12.5mg/kg, p.o.) with carbidopa (3.125, 6.25, 9.375 or 12.5mg/kg, p.o.) concomitantly, significantly increased locomotor activity compared to L-DOPA alone. There was no difference in total AUC locomotor response between any of the L-DOPA with carbidopa doses regimens.

The administration of L-DOPA (12.5mg/kg, p.o.) with carbidopa (3.125, 6.25, 9.375 or 12.5mg/kg, p.o.) concomitantly, significantly reversed motor disability compared to L-DOPA alone. There was no difference in total AUC motor disability scores between any of the L-DOPA with carbidopa doses regimens (figure 4.1B, D & F).

The administration of L-DOPA (12.5mg/kg, p.o.) with carbidopa (3.125, 6.25, 9.375 or 12.5mg/kg, p.o.) concomitantly, significantly increased dyskinesia compared to L-DOPA alone. Increasing the dose of carbidopa produced a dose related increase in dyskinesia scores with maximum effect at 12.5mg/kg.

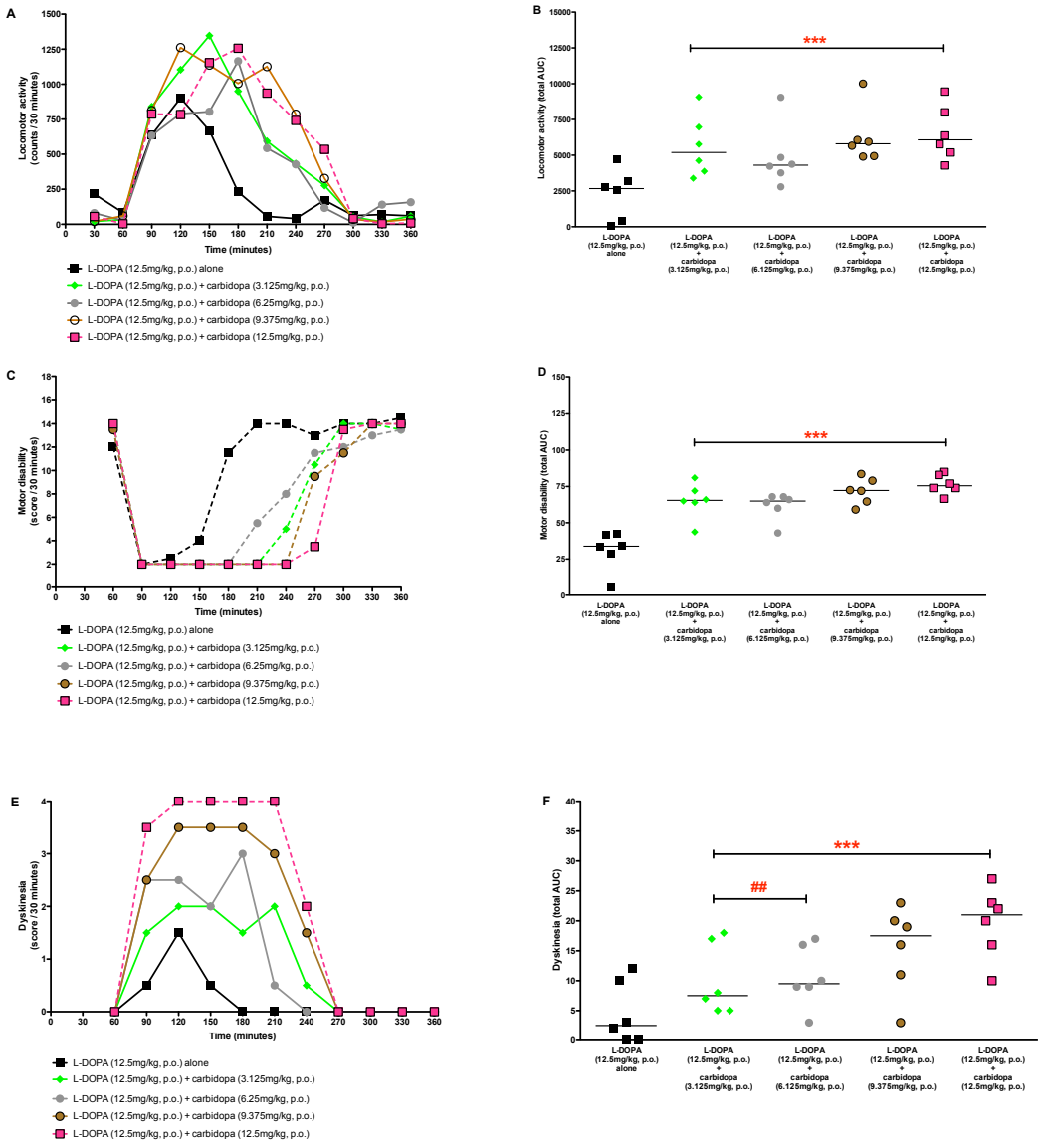


Figure 4. 1 Carbidopa dose response study in the MPTP treated common marmoset to assess the effect of L-DOPA induced locomotor activity (A&B), motor disability (C&D) and dyskinesia (E&F)

MPTP treated marmosets (n=6) were placed into test cages at t= 0 minutes and baseline behaviour assessed. At 60 minutes animals were treated with L-DOPA (12.5mg/kg, p.o.) alone or L-DOPA (12.5mg/kg, p.o.) with carbidopa (3.125, 6.25,9.375 or 12.5mg/kg, p.o.) and were observed for a further 5 hours. Data are expressed as time courses (A, C and E) for locomotor activity, motor disability and dyskinesia respectively, shown as median values with error bars omitted for clarity. Total areas under the curve (AUC) are shown (B, D and F) as individual values for animals with the median value indicated for locomotor activity, motor disability and dyskinesia respectively. *** $P < 0.001$ compared to L-DOPA (12.5mg/kg, p.o.) alone, ## $P < 0.01$ comparing L-DOPA (12.5mg/kg, p.o.) with carbidopa (3.125 or 6.25mg/kg, p.o.) to L-DOPA with carbidopa (12.5mg/kg, p.o.), repeated measures ANOVA followed by Bonferroni multi comparisons post hoc test on transformed data, $y=\sqrt{y}$.

4.8.1.2 Benserazide dose response study

The administration of L-DOPA (12.5mg/kg, p.o.) with benserazide (3.125, 6.25 or 9.375mg/kg, p.o.) concomitantly, significantly increased locomotor activity compared to L-DOPA alone. The highest dose of benserazide (12.5mg/kg) given with L-DOPA produced locomotor activity not significantly to L-DOPA alone. There was no difference in total AUC locomotor response between any of the L-DOPA with benserazide dose regimens. The administration of L-DOPA (12.5mg/kg, p.o.) with benserazide (3.125, 6.25, 9.375 or 12.5mg/kg, p.o.) concomitantly, significantly reversed motor disability compared to L-DOPA alone. There was no difference in total AUC motor disability scores between any of the L-DOPA with benserazide dose regimens.

The administration of L-DOPA (12.5mg/kg, p.o.) with benserazide (3.125, 6.25, 9.375 or 12.5mg/kg, p.o.) concomitantly, significantly increased dyskinesia compared to L-DOPA alone. The lowest dose of benserazide (3.125mg/kg) used did not produce dyskinesia that was significantly different to L-DOPA alone. Increasing the dose of benserazide produced a dose response increase in dyskinesia levels. Increasing the dose of carbidopa produced a dose related increase in dyskinesia scores with maximum effect at 12.5mg/kg (figure 4.2E & F).

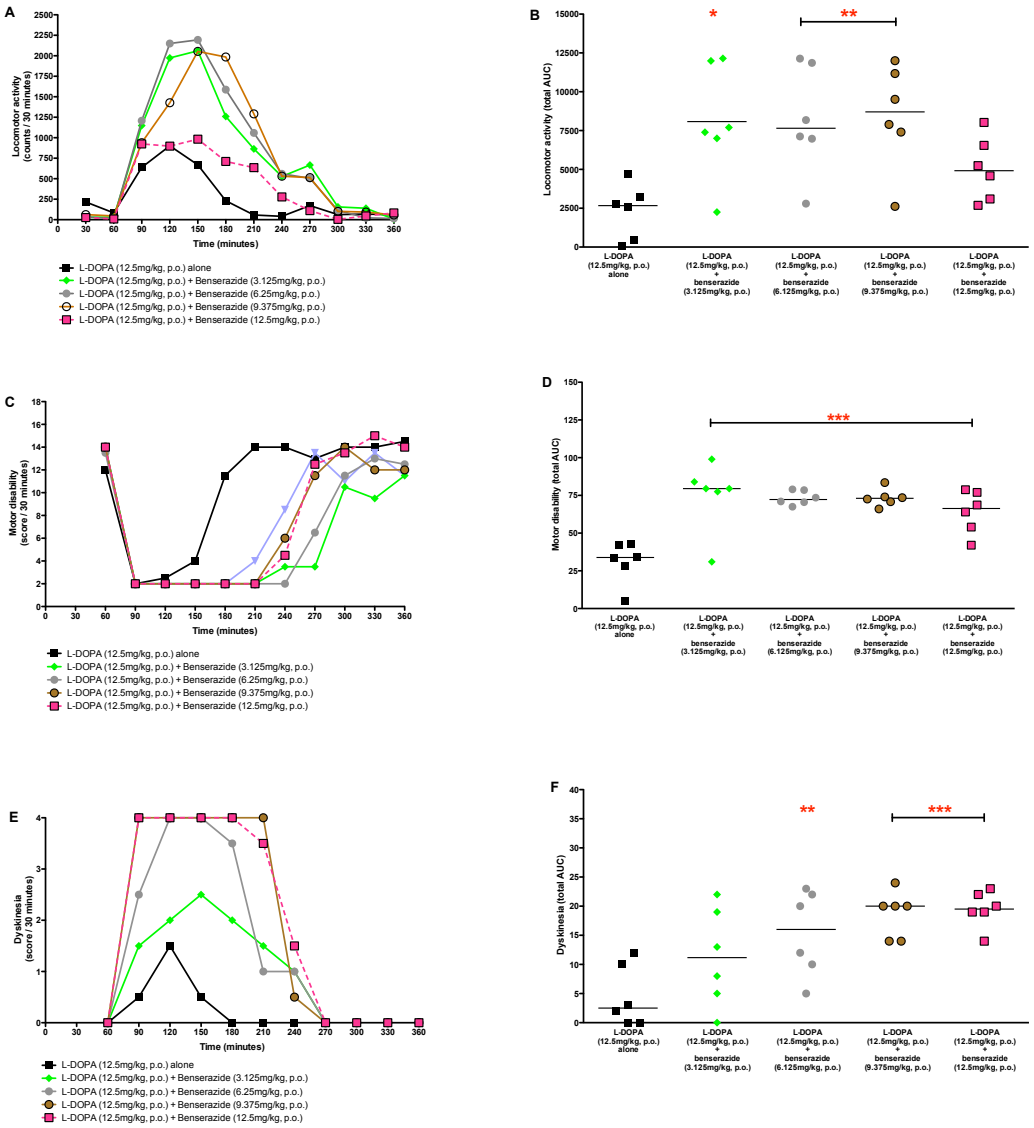


Figure 4. 2 Benserazide dose response study in MPTP treated common marmosets to assess the effect of L-DOPA induced locomotor activity (A&B), motor disability (C&D) and dyskinesia (E&F)

MPTP treated marmosets (n=6) were placed into test cages at t= 0 minutes and baseline behaviour assessed. At 60 minutes animals were treated with L-DOPA (12.5mg/kg, p.o.) alone or L-DOPA (12.5mg/kg, p.o.) with benserazide (3.125, 6.25,9.375 or 12.5mg/kg, p.o.) and were observed for a further 5 hours. Animals were given at least 3 days washout between different treatments. Data are expressed as time courses (A, C and E) for locomotor activity, motor disability and dyskinesia respectively, shown as median values with error bars omitted for clarity. Totals areas under the curve (AUC) are shown (B, D and F) as individual values for animals with the median value indicated for locomotor activity, motor disability and dyskinesia respectively. *** $P < 0.001$, ** $P < 0.01$ or *$P<0.05$ compared to L-DOPA (12.5mg/kg, p.o.) alone, repeated measures ANOVA followed by Bonferroni post hoc test on transformed data, $y=\sqrt{y}$.

4.8.1.3 Comparison of carbidopa and benserazide dose response studies

The administration of L-DOPA with either carbidopa or benserazide at all dose levels (3.125, 6.25, 9.375 and 12.5mg/kg) significantly increased locomotor activity compared to L-DOPA alone. Although not significantly different benserazide administration with L-DOPA at all doses except 12.5mg/kg produced a greater peak locomotor activity despite having no increase in duration.

The administration of L-DOPA with either carbidopa or benserazide at all dose levels (3.125, 6.25, 9.375 and 12.5mg/kg) significantly improved motor disability compared to L-DOPA alone.

The administration of L-DOPA with either carbidopa or benserazide at all dose levels (3.125, 6.25, 9.375 and 12.5mg/kg) significantly induced more dyskinesia than L-DOPA alone except for L-DOPA administered with benserazide (3.125mg/kg) which did not produce dyskinesia significantly different to L-DOPA alone.

The comparative log dose responses (figure 4.3) plotted from the two separate dose response studies shows that there is no significant difference between the effects of carbidopa and benserazide on locomotor activity, motor disability reversal and dyskinesia expression (figure 4.3A-C). However, there is a significant effect from the dose of carbidopa and benserazide that induce the L-DOPA behavioural response in terms of locomotor activity, motor disability and dyskinesia (figure 4.3A-C).

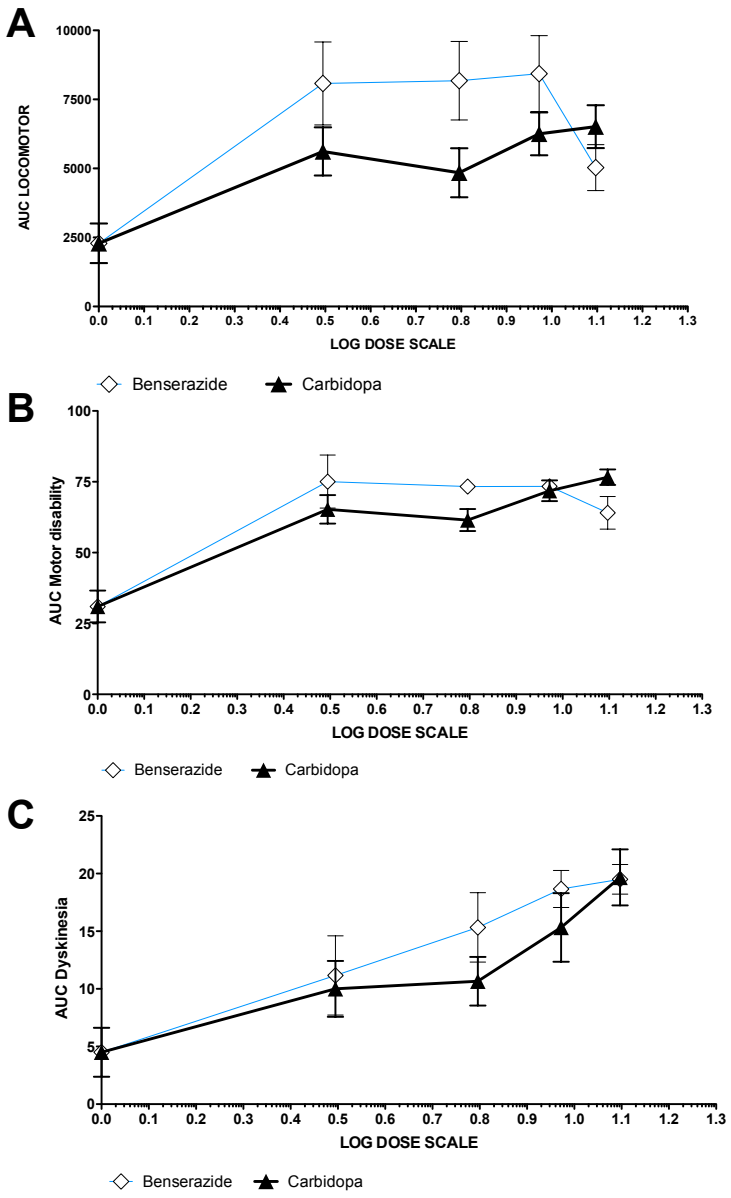


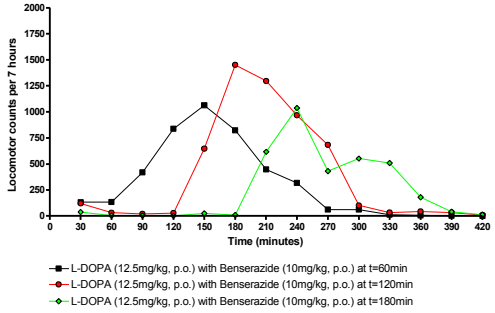
Figure 4. 3 Comparison of carbidopa and benserazide dose response studies in MPTP treated common marmosets to assess the effect of L-DOPA induced locomotor activity (A), motor disability (B) and dyskinesia (C)

MPTP treated marmosets (n=6) were placed into test cages at t= 0 minutes then at 60 minutes animals were treated with L-DOPA (12.5mg/kg, p.o.) alone or L-DOPA (12.5mg/kg, p.o.) with benserazide or carbidopa (3.125, 6.25,9.375 or 12.5mg/kg, p.o.) and were observed for a further 5 hours. Animals were given at least 2 days washout between different treatments. Data are expressed log dose responses based on total AUC. Statistical analysis with 2 way ANOVA showed no significant difference ($P > 0.05$) between benserazide and carbidopa

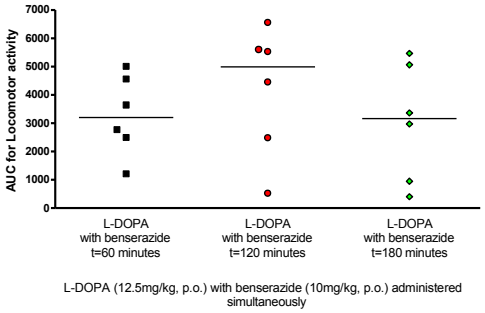
4.8.2 Investigation of the effect of acclimatisation on motor function induced by L-DOPA and benserazide administration in the MPTP treated marmoset

The administration of L-DOPA (12.5mg/kg, p.o.) with benserazide (10mg/kg, p.o.) concomitantly at 60, 120 and 180 minutes induced an increase in locomotor activity, reversed motor disability and induced marked to severe dyskinesia (Figure 4.4). Administration of this treatment regimen at different time points did not significantly alter the L-DOPA induced response for locomotor activity, motor disability or dyskinesia (Figure 4.4). At the time points of drug administration used in this study, there were no significant differences in the motor response to L-DOPA administration with benserazide.

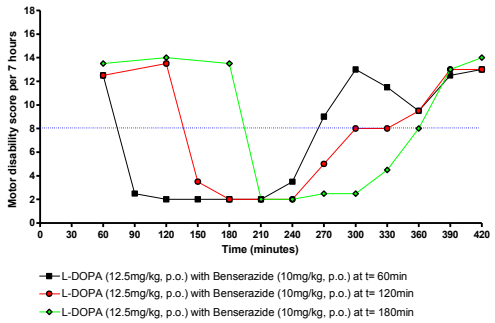
A


Locomotor counts per 7 hours
Time (minutes)
L-DOPA (12.5mg/kg, p.o.) with Benserazide (10mg/kg, p.o.) at t=60min
L-DOPA (12.5mg/kg, p.o.) with Benserazide (10mg/kg, p.o.) at t=120min
L-DOPA (12.5mg/kg, p.o.) with Benserazide (10mg/kg, p.o.) at t=180min

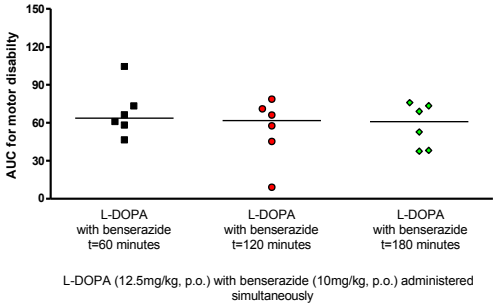
B


AUC for Locomotor activity
L-DOPA with benserazide t=60 minutes
L-DOPA with benserazide t=120 minutes
L-DOPA with benserazide t=180 minutes
L-DOPA (12.5mg/kg, p.o.) with benserazide (10mg/kg, p.o.) administered simultaneously

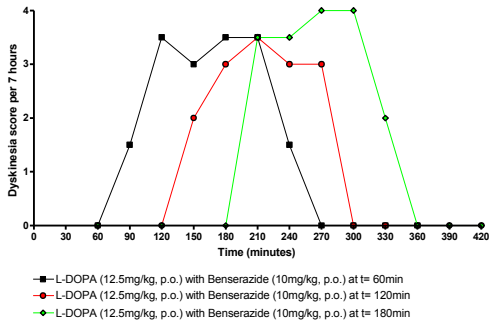
C


Motor disability score per 7 hours
Time (minutes)
L-DOPA (12.5mg/kg, p.o.) with Benserazide (10mg/kg, p.o.) at t= 60min
L-DOPA (12.5mg/kg, p.o.) with Benserazide (10mg/kg, p.o.) at t= 120min
L-DOPA (12.5mg/kg, p.o.) with Benserazide (10mg/kg, p.o.) at t= 180min

D


AUC for motor disability
L-DOPA with benserazide t=60 minutes
L-DOPA with benserazide t=120 minutes
L-DOPA with benserazide t=180 minutes
L-DOPA (12.5mg/kg, p.o.) with benserazide (10mg/kg, p.o.) administered simultaneously

E


Dyskinesia score per 7 hours
Time (minutes)
L-DOPA (12.5mg/kg, p.o.) with Benserazide (10mg/kg, p.o.) at t= 60min
L-DOPA (12.5mg/kg, p.o.) with Benserazide (10mg/kg, p.o.) at t= 120min
L-DOPA (12.5mg/kg, p.o.) with Benserazide (10mg/kg, p.o.) at t= 180min

F

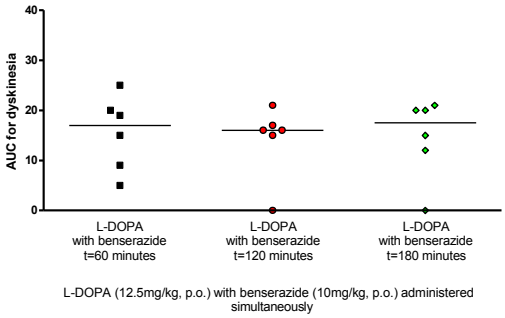

AUC for dyskinesia
L-DOPA with benserazide t=60 minutes
L-DOPA with benserazide t=120 minutes
L-DOPA with benserazide t=180 minutes
L-DOPA (12.5mg/kg, p.o.) with benserazide (10mg/kg, p.o.) administered simultaneously

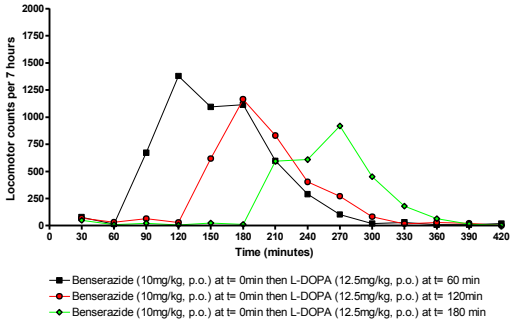
Figure 4. 4 Investigation of the effect of acclimatization on motor function induced by L-DOPA and benserazide administration in the MPTP treated common marmoset

MPTP treated marmosets (n=6) were placed into test cages at t= 0 minutes and baseline locomotor activity, motor disability and dyskinesia were recorded for 60 minutes then at 60, 120 or 180 minutes animals were treated with L-DOPA (12.5mg/kg, p.o.) with benserazide (10mg/kg, p.o.) and were observed for a further 6 hours. Animals were given at least 3 days washout between different treatments. Data are expressed as time courses (A, C and E) for locomotor activity, motor disability and dyskinesia respectively shown as median values with error bars omitted for clarity. Totals areas under the curve (AUC) are shown (B, D and F) as individual values for animals with the median value indicated (P = 0.56, 0.56 and 0.85 respectively). $P > 0.05$ compared to L-DOPA (12.5mg/kg, p.o.) with benserazide (10mg/kg, p.o.) dosed at t=60 minutes, repeated measures ANOVA followed by Dunnett's post hoc test on transformed data, $y=\sqrt{y}$.

4.8.2.1 Investigation of the effect of timing of dopa decarboxylase inhibitor administration

Administration of benserazide (10mg/kg, p.o.) as pre-treatment prior to L-DOPA (12.5mg/kg, p.o.) at 3 different time points (60, 120 or 180 minutes) produced no significant difference in terms of locomotor activity, motor disability or dyskinesia. Although not significant, there was a trend for L-DOPA induced locomotor activity and motor disability reversal to be greater with L-DOPA administration at 60 minutes rather than the later time points. Benserazide is still present in the marmoset even after 3 hours as there was no significant difference in the L-DOPA induced response compared to earlier time points. Whilst benserazide has not been directly measured at these time points, its presence is inferred from L-DOPA induced activity and the fact that total AUC between the 3 time points is not significantly different.

A



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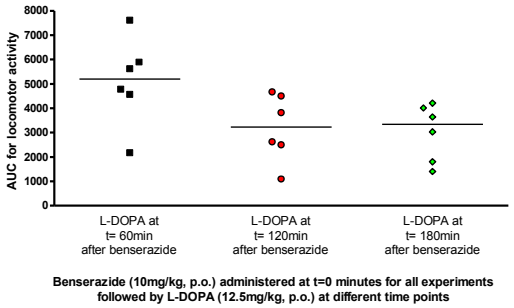
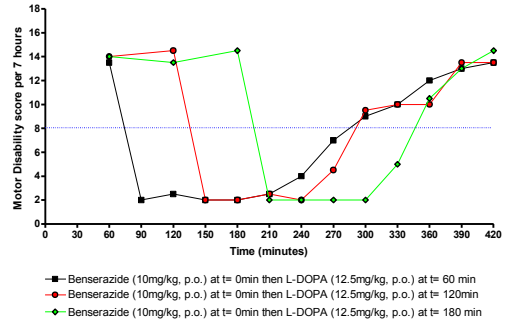


Benserazide (10mg/kg, p.o.) administered at t=0 minutes for all experiments followed by L-DOPA (12.5mg/kg, p.o.) at different time points

C



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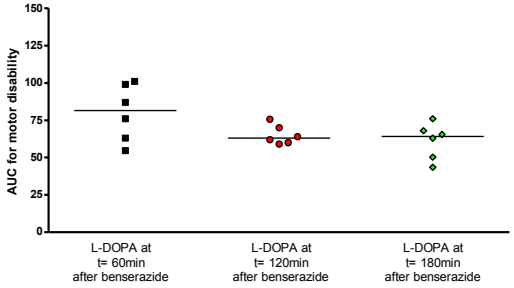
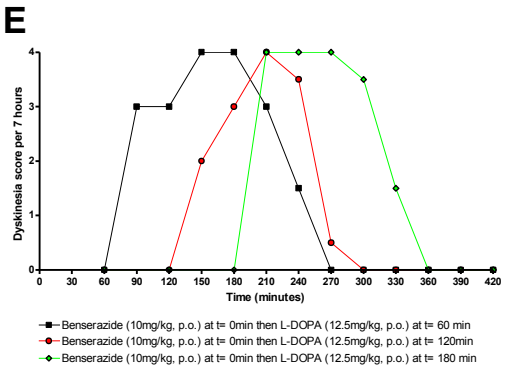


Benserazide (10mg/kg, p.o.) administered at t=0 minutes for all experiments followed by L-DOPA (12.5mg/kg, p.o.) at different time points

E



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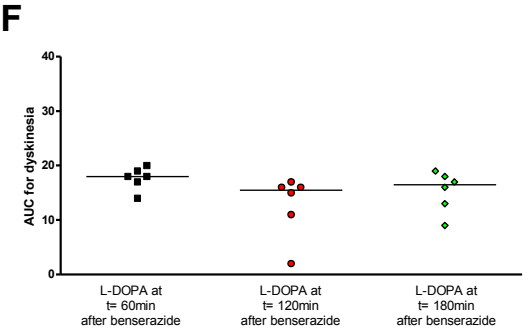


Benserazide (10mg/kg, p.o.) administered at t=0 minutes for all experiments followed by L-DOPA (12.5mg/kg, p.o.) at different time points

Figure 4. 5 Investigation of the effect of timing of dopa decarboxylase inhibitor administration in MPTP treated common marmosets

MPTP treated marmosets (n=6) were treated with benserazide (10mg/kg, p.o) and placed into test cages at t= 0 minutes and baseline locomotor activity, motor disability and dyskinesia were recorded for 60 minutes then at 60, 120 or 180 minutes animals were treated with L-DOPA (12.5mg/kg, p.o.) and were observed for a further 6 hours. Animals were given at least 3 days washout between different treatments. Data are expressed as time courses (A, C and E) for locomotor activity, motor disability and dyskinesia respectively shown as median values with error bars omitted for clarity. Totals areas under the curve (AUC) are shown (B, D and F) as individual values for animals with the median value indicated. $P > 0.05$ compared to L-DOPA (12.5mg/kg, p.o.) with benserazide (10mg/kg, p.o.) dosed at t=60 minutes, repeated measures ANOVA followed by Dunnett's post hoc test on transformed data, $y=\sqrt{y}$.

4.8.3 Investigation of the effects of L-AMD on L-dopa induced motor function

The administration of L-DOPA (12.5 mg/kg) with L-AMD (12.5 mg/kg) produced a significant increase in locomotor activity and improved motor disability reversal compared to L-DOPA (12.5 mg/kg) alone (Figure 4.6). There was no significant difference between L-DOPA induced reversal of motor deficits with carbidopa, benserazide or L-AMD. For comparative purposes, data from the carbidopa and benserazide dose response studies has been utilized to compare with the effects of L-AMD. The administration of L-DOPA with either carbidopa or benserazide resulted in significantly greater levels of dyskinesia compared to L-DOPA alone (Figure 4.6). In contrast, a combination of L-DOPA with L-AMD did not increase dyskinesia compared to that produced by L-DOPA alone in these animals which had previously been treated with L-DOPA to induce dyskinesia expression (Figure 4.6) and L-DOPA with L-AMD combination produced significantly less dyskinesia than observed with L-DOPA with either carbidopa or benserazide (Figure 4.6).

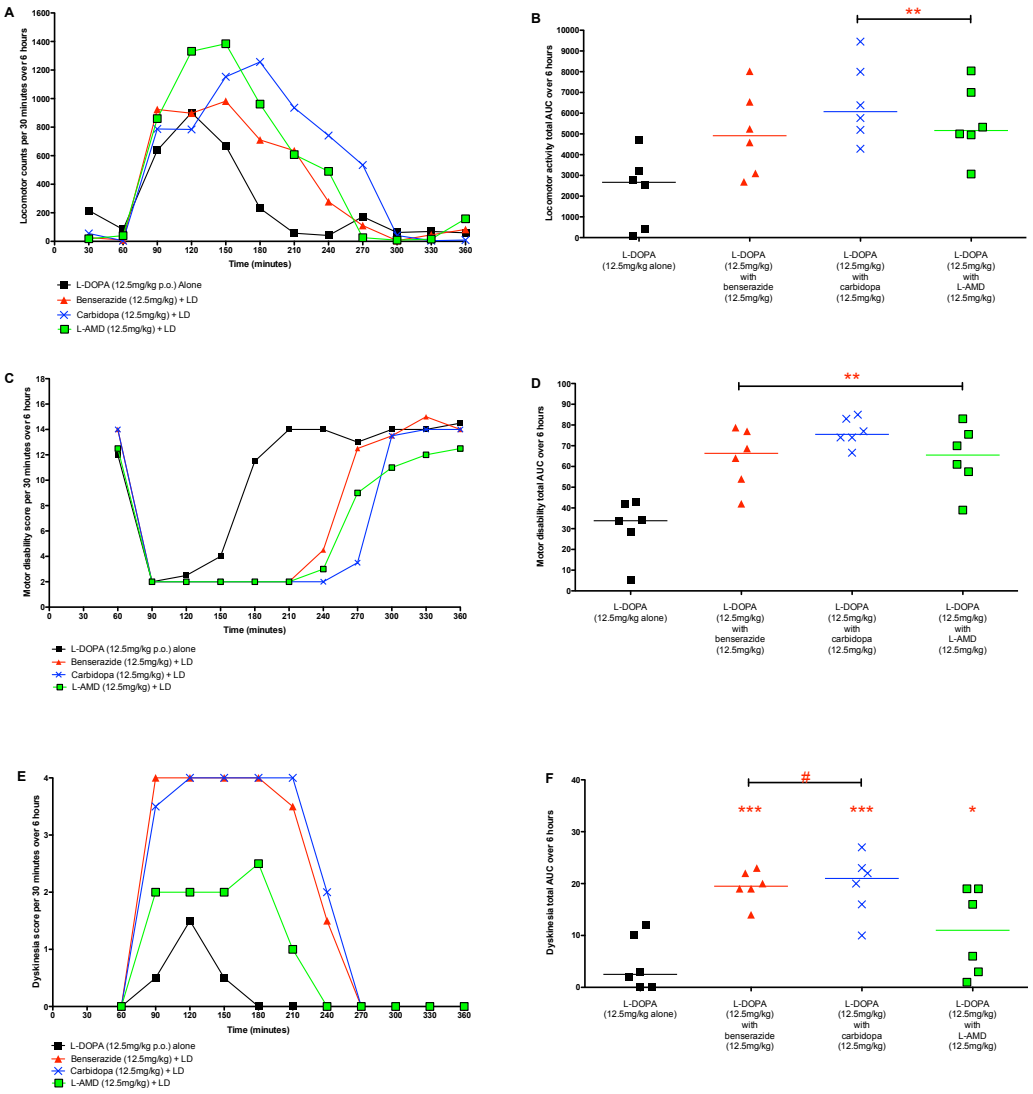


Figure 4. 6 Comparison of carbidopa, benserazide and L-AMD in MPTP treated common marmosets to assess the effect of L-DOPA induced locomotor activity, motor disability and dyskinesia timecourses (A,C & E) and totals (B, D & F)

MPTP treated marmosets (n=6) were placed into test cages at t= 0 minutes and baseline locomotor activity was recorded for 60 minutes then at 60 minutes animals were treated with L-DOPA (12.5mg/kg, p.o.) alone, L-DOPA (12.5mg/kg, p.o.) with benserazide or carbidopa or L-AMD (12.5mg/kg, p.o.; dose chosen from chemical structure similarity to other DDCIs) and were observed for a further 5 hours. Animals were given at least 3 days washout between different treatments. Data are expressed as time courses (A, C and E), shown as median values with error bars omitted for clarity. Totals areas under the curve (AUC) are shown (B, D and F) as individual values for animals with the median value indicated for locomotor activity, motor disability and dyskinesia respectively. *** $P < 0.001$ or ** $P < 0.01$ or * $P < 0.05$ compared to L-DOPA (12.5mg/kg, p.o.) alone, # $P < 0.05$ compared to L-DOPA with L-AMD, repeated measures ANOVA followed by Bonferroni post hoc test on transformed data, $y=\sqrt{y}$.

4.9 Discussion

In preclinical studies as well as in a clinical setting, dopa-decarboxylase inhibitors are employed on a routine basis to potentiate the effects of L-DOPA's actions on motor function (Kaakkola et al. 1990). But until the current studies undertaken in this thesis there does not appear to have been any attempt to optimise the combinations employed or to determine the optimal timings of treatment and the doses of dopa-decarboxylase inhibitors to reduce dyskinesia whilst providing an effective reversal of motor disability. The pre-clinical use of DDCIs is usually based on prior literature and are historical in nature or they use ratios pre-determined in clinically used tablets, adjusting the dose of both drugs based on the L-DOPA requirements only (i.e. cut a pill in half to get half the L-DOPA dose).

The studies demonstrated in this thesis have attempted to investigate whether manipulation of the use of dopa-decarboxylase inhibitors offers improved L-DOPA responses in predictive animal models of disease, in the hope of optimising current Parkinson's disease treatments and improving patient outcomes. In the previous Chapter, we showed the importance of timing of administration of benserazide in the 6-OHDA lesioned rat. The current studies were carried out in the MPTP-treated primate model of Parkinson's disease, which is highly predictive of drug action in man and involved a comparison between carbidopa and benserazide which has the benefit of measuring motor disability and dyskinesia, in a clinically relevant manner (Eslamboli. 2005). The first set of experiments set out to evaluate whether the timing / acclimatisation of concomitant L-DOPA and

benserazide administration in the morning period influenced the L-DOPA induced response. The results showed that over the three-hour period in the morning, there was no difference in L-DOPA induced locomotor activity, reversal of motor disability and expression of dyskinesia. The results show here that there is a difference in response between 6-OHDA lesioned rats and the MPTP treated common marmoset in relation to impact of acclimatisation. The results in the MPTP model correlate more closely to that seen in man (Morin et al. 2013). This could be due to the rate of gastric emptying which naturally occurs and would not be significantly different in that morning period in man and primate but would be different to that of rodents which are nocturnal animals and would be experiencing changes of gastric emptying and also circadian rhythm of natural dopamine turnover (Bonuccelli et al. 2000).

The second component of the study related to the time course of dopa-decarboxylase inhibitors effect relative to that of L-DOPA. Two major questions were addressed which apply largely to the use of dopa-decarboxylase inhibitors in experimental studies, but to some extent to their effect in clinical practice. This relates to the pharmacokinetics of dopa-decarboxylase inhibitors as evidenced in the literature (Schwartz et al. 1974) and whether effective plasma levels persist throughout the potential time course of activity of L-DOPA in terms of motor behaviour. Statistically there did not appear to be any difference whether L-DOPA was administered 60 min or 180 min after benserazide was administered, suggesting the decarboxylase was adequately inhibited throughout the

testing period. Certainly an acclimatisation period seemed to have no effect on the motor response to L-DOPA given with benserazide.

Surprisingly, there has been little investigation of the pharmacokinetic profile of carbidopa or benserazide. Early studies suggested that the half-life of both carbidopa and benserazide exceeds that of L-DOPA (2–3 h vs. 90 min) and that would be consistent with the findings of this investigation. One confounding factor is that benserazide may act through an active metabolite (Ro 04-5127) whose pharmacokinetic profile appears unknown (Da et al. 1987; Grange et al. 2001). The next part of the study dealt with the question of dose of dopa-decarboxylase inhibitors employed. Again there has been relatively little investigation of this either in experimental studies or in clinical practice and it is difficult to determine how doses were derived in either case. In these studies a high dose of dopa-decarboxylase inhibitors (12.5 mg/kg) was used to ensure that there was a maximal inhibition of DDC. The first point made by the study is that in the doses routinely employed in MPTP-treated primates, L-DOPA alone exerts a significant effect on motor function as it did in the early era of its use in the treatment of Parkinson's disease (Marsden et al. 1973c; Greenacre et al. 1976). Indeed, the overall effect of dopa-decarboxylase inhibition in the MPTP-treated primate is to increase the duration of effect of L-DOPA.

Carbidopa has been routinely employed at a dose of 12.5 mg / kg p.o. in studies undertaken in these laboratories and elsewhere. These studies show that carbidopa is highly effective in lower doses but that its ability to

prolong the duration of effect of L-DOPA is dose related when using a high dose L-DOPA (12.5 mg/kg). This suggests either that inhibition of decarboxylase activity is not complete at lower doses or that the greater plasma levels of carbidopa at higher doses stay in the effective range for the inhibition of enzyme activity for a longer period of time. What was most obvious, however, was that its effect on dyskinesia expression was closely dose related and involuntary movements appeared to increase in severity even when motor disability was not being further enhanced by increasing dopa-decarboxylase inhibitor dose. It could be, however, that the increasing intensity of dyskinesia was inhibitory on the rating of motor disability and impaired controlled locomotor activity. Benserazide has been used in doses of 1–50 mg/kg p.o in previous studies in MPTP-treated primates but its effects do not appear as dose related as those of carbidopa and higher doses may be inhibitory on the reversal of locomotor activity and motor disability (Pearce et al. 1995; Quik et al. 2002). Again dyskinesia intensity appeared to increase in a dose related manner and to continue to advance while motor disability either remained stable or worsened. The reasons for this pattern of activity need further investigation but could relate to penetration of higher doses of benserazide in to the brain so inhibiting central decarboxylase activity although this would not explain the increase in dyskinesia. Indeed, the direct injection of benserazide into the striatum inhibited L-DOPA induced dyskinesia in the rat AIMs model (Buck et al. 2008).

Another explanation of these results could be that at these high doses of both L-DOPA and DDCIs animals cannot be more active or motor disability

more reversed (limited scoring scale; refer to section 2.3) but the increase in dopamine levels in the brain can result in greater dyskinesia expression. The intense dyskinesia seen with higher doses, suggests a greater effect of L-DOPA derived dopamine in brain although disruption of the behavioural assessment might be impaired by the dyskinetic movements. There have been many studies whereby carbidopa and benserazide have been examined *in-vitro* and also in a clinical setting and the conclusions that were drawn seemed to show that there is no difference between the two dopa-decarboxylase inhibitors as was confirmed in these studies. However, most of the studies did not use molar equivalent doses of the compounds. In studies carried out in our labs with the use of the irreversible central dopa-decarboxylase inhibitor (NSD-1015) (Treseder et al. 2000) it was shown that the compound alone induced vomiting and attenuated L-DOPA's activity on motor function. Treseder et al also showed that dopa-decarboxylase inhibitors could inhibit MAO-B activity and this could indeed play some role in the effects observed in the drug and was able to penetrate the blood brain barrier.

Another possible explanation for these results could be in the metabolism of L-DOPA via COMT. From the literature and *invitro* studies, it has been shown that both carbidopa and benserazide have an effect on COMT (Boomsma et al. 1986). Whilst it has been shown clinically that the use of COMT inhibitors such as entacapone (Brooks et al. 2008) offer greater benefit that standard L-DOPA plus DDCI formulation, it has not been shown whether high residual doses of DDCI, due to multiple daily doses have an effect on the COMT metabolism of L-DOPA. It could be that at these high

doses of DDCI, there is an enhanced COMT inhibitory effect which again results in greater brain levels of L-DOPA derived dopamine, which causes more pulsatile and erratic dopaminergic stimulation and hence greater dyskinesia expression.

This investigation set out to look at the factors that affect the ability of dopa-decarboxylase inhibitors to potentiate L-DOPA's action in reversing motor deficits in Parkinson's disease. In the MPTP-treated primate the lowest commonly used dose of carbidopa and benserazide appear to result in maximal effects and if anything, the results argue for the use of lower rather than higher dopa-decarboxylase inhibitors doses in such studies. While in this study the dose of L-DOPA was constant and deliberately chosen to be in the range causing an effect when administered alone, it would be interesting to look at the effects of a constant and maximally effective dose of dopa-decarboxylase inhibitors in conjunction with variable doses of L-DOPA to determine how this alters the motor response. The answer to this question might then determine precisely what ratio of L-DOPA to dopa-decarboxylase inhibitors should be used in clinical practice.

In the final experiment of this chapter, L-AMD was used to assess the ability of currently non-clinically used DDCI's in Parkinson's disease to have an impact on L-DOPA induced behaviour. L-AMD in place of carbidopa or benserazide did not alter locomotor activity or motor disability compared to L-DOPA plus either carbidopa or benserazide but it produced

significantly less dyskinesia when compared to carbidopa or benserazide combinations with L-DOPA (figure 4.6) or that the dose is not equivalent. In either case this indicates that new or novel inhibitors of DDC can have a potential to affect the pharmacodynamic effects of L-DOPA behaviour and even dyskinesia. A possible explanation for this could be due to penetration of L-AMD into the brain and as such prevent all the L-DOPA that has entered the CNS to be metabolised to dopamine, thus having a slower and less pulsatile dopaminergic effect (Di Stefano et al. 2008). L-AMD could be having this effect due to its greater ability to prevent decarboxylation of L-DOPA to DA in the CNS as opposed to the periphery. In the CNS, L-AMD is more efficient at binding to vitamin B6 (Hess et al. 1961) and pyridoxal phosphate enzyme substrates (compared to the peripheral nervous system). This would mean that L-AMD offers greater L-DOPA protection from metabolism by DDC. Therefore as levels of L-AMD begin to drop (due to its metabolism) L-DOPA is more smoothly converted to DA. Another possible explanation could be that L-AMD also interacts with endogenous DA more than benserazide or carbidopa. It could be that the total L-AMD effect in regulating continuous DA stimulation (both external L-DOPA administration and endogenous DA production) is responsible for the observed reduction in dyskinesia (Pinder et al. 1976). However, until more comprehensive experiments are conducted it would be difficult to understand the exact mechanism of this dyskinesia reduction.

It is not clear from the literature why carbidopa and benserazide were adopted as DDCIs so quickly in combination with L-DOPA to treat

Parkinson's disease but there use has not been strongly contested and whilst research has focused specifically on L-DOPA and dopamine agonists, the important role of the DDCI could potentially go beyond peripheral protection of L-DOPA and its associated side effects. This is an area, which requires more attention and could hold a forgotten but potential new avenue to Parkinson's disease treatment with L-DOPA

Summary

The experiments in this chapter set out to test the hypothesis that L-DOPA induced locomotor activity, motor disability and dyskinesia can be improved by optimising the dose, timing of the dopa-decarboxylase inhibitor and the use of non-clinically adopted DDCIs. The results in this chapter show that there is no significant difference between benserazide and carbidopa when used with L-DOPA but there are significant differences in L-DOPA response based on the dose of DDCI used.

This chapter demonstrated that our hypothesis is true for the dose of DDCI and the potential use of non-clinically prescribed DDCIs but not for the timing of drug administration. Therefore the beneficial use of L-DOPA is entirely dependent on the efficacy and dose of the DDCI used which raises the question of whether L-DOPA itself could be improved upon to potentially not require a drug combination with DDCIs to have a therapeutic effect or demonstrate improved behavioural responses with the existing DDCIs. The following chapter investigates a novel L-DOPA pro-drug to investigate these questions and help us to understand whether the

efficacy of L-DOPA can be improved by reducing the expression of dyskinesia whilst maintaining optimal motor function in Parkinson's disease by again utilising the predictive MPTP-treated marmoset model.

Chapter 5

The effect of modifying the structure of L-DOPA on the anti-parkinsonian effect in MPTP-treated common marmosets

5. The effect of modifying the structure of L-DOPA on the anti-parkinsonian effect in MPTP-treated common marmosets

5.1 Introduction

From the investigations in the previous chapters, we have shown that we can improve the L-DOPA behavioural effect in predictive animal models of Parkinson's disease by optimising the dose and choice of DDCIs. However, a major drawback to the use of immediate release forms of L-DOPA (Sinemet and Madopar) is the erratic absorption and subsequent rapid metabolism of L-DOPA (Pilleri & Antonini. 2014). These facts coupled to its relatively short plasma half-life leads to erratic plasma levels and fluctuating clinical response (Kuoppamäki et al. 2009). These pharmacokinetic features of L-DOPA are implicated in the development of motor fluctuations and motor complications such as dyskinesia that invariably occur on long term use of L-DOPA and that eventually becomes treatment limiting (Manson et al. 2012). Therefore, improvement in the pharmacokinetics of L-DOPA may reduce these motor complications.

One of the major bioavailability problems with L-DOPA relates to its absorption from the gut. L-DOPA is largely absorbed by active transport by a rate limiting large neutral amino acid transporter located in the upper small intestine (Del Amo et al. 2008). Consequently, this differs from how most drugs are absorbed which is by simple passive diffusion over the long

portion of the gastro-intestinal tract, with the lower third consisting of the duodenum and jejunum (Sozio et al., 2012). The implication is that L-DOPA absorption is limited by transporter availability in a short length of the GI tract and coupled with protein from food intake and considering that absorption can only occur in a specific region of the intestine results in an unpredictable L-DOPA ADME profile. It is for this reason that controlled release preparations of L-DOPA (Sinemet CR and Madopar HBS) have so far failed to achieve more prolonged delivery, as much of the drug is released too far down the intestine for absorption to occur (Nordera et al. 1987; Koller and Pahwa. 1994).

There are a variety of methods, which are being studied around drug delivery improvements utilising pro-drugs. Some of these methods include lipophilic pro-drugs, carrier mediated pro-drugs and co-drugs (Sozio et al. 2012). The lipophilic pro-drug approach is based on the inability of the active parent drug moiety to penetrate the BBB and requires a mechanism of penetration. In this situation the polar chemical elements of the compound are masked using a lipidisation method which allows the pro-drug to have an enhanced ability to cross the BBB and then be enzymatically cleaved to form the active drug molecule(s) (Oldendorf et al. 1972). However, L-DOPA can both cross the gut and the BBB, so this is not a useful approach in this situation.

One approach would be the development of a pro-drug that protects the metabolism of L-DOPA and indeed Felix and colleagues examined the

effects of a series of di- and tripeptides derivatives of L-DOPA in mice (Felix et al. 1974). The derivatives were developed from decades of L-DOPA pro-drug investigations, which focused on systemically protecting the main sites of L-DOPA metabolism including the carboxy function, the amino and catechol system. Despite initial optimism about some of the PK results, the compounds failed to show more potency or longer duration of action than L-DOPA itself. The dimeric approach has since been evaluated with L-DOPA covalently bonded to benserazide (Di Stefano et al. 2006), which still failed to show any further improvement over standard L-DOPA.

Another approach is carrier-mediated pro-drugs. These pro-drugs have an additional endogenous substrate attached to their chemical structure, which is recognized by transporters on the BBB transport system which allows easier translocation to the required sites in the brain (Ohtsuki et al. 2007). The approach of co-drugs whereby 2 active drug molecules are combined together and once passed the BBB are cleaved and have complimentary effects on each other, enhancing efficacy (Cavalli et al. 2008).

There is a clear division between improving delivery of L-DOPA and enhancement of L-DOPA efficacy via pro-drug strategies. One option is to combine these strategies in a single pro-drug.

PRX 1354 (figure 5.0 A) is a pro-drug derivative of L-DOPA that has been designed to improve solubility, increase stability and to provide an extended duration of drug presence in plasma by avoiding absorption by

the amino acid transporter and utilising simple passive diffusion to enter the body. It was designed to avoid metabolism of peripheral DDC and to degrade slowly to L-DOPA and in so doing, extend the duration of plasma L-DOPA concentration after each dose. Because of patent issues and on-going R&D developments around the compound, the functional groups added at R1 to R4 cannot be identified (figure 5.0 A).

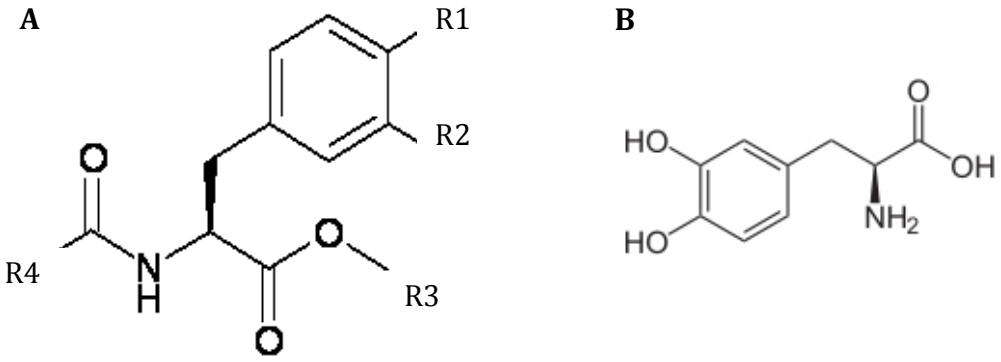


Figure 5.0 Chemical structures of PRX 1354 (A) and L-DOPA (B)

The chemical manipulations that have altered L-DOPA to a pro-drug are situated in positions R1-R4.

In essence R1 and R2 are attached to the catechol moiety of L-DOPA and serve to reduce its polarity and increase lipid solubility. R3 and R4 are bound to the carboxylic acid and amino groups respectively. They remove affinity for the amino acid transporter, protect the carboxylic acid group from enzymatic removal by DDC and also serve to further increase lipid solubility and to promote passive absorption. The structure of R4 also contained an L-DOPA moiety. Therefore PRX1354 in essence is designed as

a double L-DOPA di-peptide molecule which when cleaved with a theoretical ability to create twice as much L-DOPA *invivo*. The theory was that once absorbed the drug would be rapidly metabolised by catalytic protease enzymes to release one molecule of L-DOPA from R4 to provide an immediate response but that the resulting metabolite was then slowly degraded to release a further L-DOPA molecule over a longer period of time thereby sustaining L-DOPA plasma levels and producing a more continuous motor response (Brod et al. 2012).

Nevertheless, the ideal L-DOPA prodrug able to effectively simulate normal physiological striatal dopamine levels, offer long term adherence incentives, delay the narrowing of the therapeutic window for L-DOPA and even potentially slow the progression of the disease, still remains to be identified (Sozio et al. 2012).

As a result of being able to improve L-DOPA efficacy by optimising the delivery of the molecule by pro-drug formation, this chapter focuses on optimising the L-DOPA effect on motor disability and dyskinesia in the MPTP treated common marmoset.

The aim for this chapter was to assess whether PRX 1354 provided an effective prodrug approach to reversing motor symptoms compared to L-DOPA, by examining its effects in the MPTP treated marmoset alone and in combination with carbidopa or benserazide.

Thus it was hypothesised that the efficacy of L-DOPA could be improved, and the fluctuations reduced by altering the chemical formulation of L-

DOPA to a dipeptide prodrug (PRX 1354) ultimately containing two moieties of L-DOPA.

5.2 Aims

In order to test this hypothesis, studies were performed in MPTP-treated common marmosets with the following aims:

a) To examine whether PRX 1354 will exert an anti-parkinsonian effect without concomitant peripheral dopa-decarboxylase inhibitor treatment.
b) To determine if the effects of PRX 1354 improve when administered with either carbidopa or benserazide
c) To compare the dose-related efficacy of PRX 1354 with molar equivalent doses of L-DOPA on the reversal of motor deficits and dyskinesia expression
d) To determine if repeated administration of PRX 1354 produces an enhanced improvement in locomotor activity, motor disability and dyskinesia expression compared to three times daily administration of the molar equivalent of L-DOPA

5.3 Methods and materials

A brief overview of the materials and methods used in the studies reported in this chapter are given here but the detailed methodology is described in Chapter 2.

5.3.1 Animals

Male and female adult common marmosets (350g or above) (Harlan UK) were given two weeks to acclimatize prior to MPTP treatment. Animals were housed either in single cages or in male and female pairs providing the male was vesectomised. Animals were placed in holding rooms which operate on a 12 hour light / dark cycle, 50% humidity at a temperature of 25 ± 1. Animals had *ad libutum* access to Mazuri pellets and water at all times. For details of MPTP treatment please refer to section 2.3. Following MPTP treatment, animals were allowed 8-12 weeks for motor deficits to stabilize and then primed with L-DOPA plus carbidopa unless stated other wise in the figure legend. On test days, animals were given at least 60 minutes acclimatization in the test cage prior to drug treatment unless stated in the figure legend. The animals used in the studies described in this chapter were L-DOPA primed and had received previous drug treatments. Animals had over 4 weeks wash out period prior to starting in these studies.

5.3.2 Drugs

PRX 1354 (supplied by Proximagen Neuroscience Plc.), carbidopa and benserazide were dissolved in 0.9% saline and given orally by gavage in a volume of 2.0ml/kg. L-DOPA was made up in acidified saline and given orally by gavage in a volume of 2.0ml/kg.

5.3.3 Statistical analysis

All statistical analysis was carried out on total AUC data only and not on time course data. Total AUC data was transformed using the command y= $\sqrt{}$y and analysed. Detailed explanations of the statistical analysis used in this chapter are described in Chapter 2.

5.4 The effect of PRX 1354 (9.8mg/kg, molar equivalent of L-DOPA 4mg/kg) administered either alone, with benserazide (10mg/kg) or with carbidopa (12.5mg/kg)

MPTP treated common marmosets (n=5) were removed from their home cages and their body weight recorded. Animals were then placed into locomotor activity test units. The MPTP treated marmosets were assessed over a 60-minute acclimatisation period to provide a baseline activity data. They were then dosed with PRX 1354 (9.8mg/kg, p.o.) alone or in combination with either benserazide (10mg/kg, p.o.) or carbidopa (12.5mg/kg) then placed back into the test units. A repeated cross over design was used with at least 4 days washout prior to the next drug treatment (i.e. over 3 days, each treatment group had n=2 animals).

Throughout the test period of 300 minutes, animals were continuously monitored for locomotor activity and scored for motor disability and dyskinesia according to previously reported scales (Pearce et al. 1995) as described in chapter 2. Scoring took place during the last 10 minutes of every half hour. Data plotted as a time course of effect and Area Under the Curve (AUC) was calculated as described in section 2.4.2.

	Time course of activity	
	0 - 60 mins	60 - 300 mins
Group 1	Baseline	PRX1354 (9.8mg/kg) + benserazide (10mg/kg)
Group 2	Baseline	PRX1354 (9.8mg/kg) + carbidopa (12.5mg/kg)
Group 3	Baseline	PRX1354 (9.8mg/kg) + vehicle

5.5 Comparison of the dose-related effect of PRX 1354 administered with benserazide (10mg/kg) compared to molar equivalent doses of L-DOPA (2, 4 and 8mg/kg) plus benserazide (10mg/kg) in MPTP treated common marmosets

MPTP treated common marmosets (n=6) were removed from their home cages and their body weight recorded. Animals were then placed into locomotor activity test units. The MPTP treated marmosets were assessed over a 60-minute acclimatisation period to provide a baseline activity data. Animals were dosed with either L-DOPA (2.0, 4.0 or 8.0mg/kg, p.o.) with benserazide (10mg/kg, p.o.) concomitantly or PRX 1354 at the molar equivalent concentration to L-DOPA (4.9, 9.8 and 19.6mg/kg, p.o., respectively) plus concomitant benserazide (10mg/kg). They were monitored for locomotor activity, motor disability and dyskinesia over 420

minutes as described in detail in Chapter 2. Each dose was tested on three separate occasions to assess the consistency of response to PRX 1354 compared to L-DOPA and the results presented are the mean response of the three dose challenges.

	Time course of activity	
	0 - 60 mins	60 - 420 mins
Group 1	Baseline	L-DOPA (2mg/kg) + benserazide (10mg/kg)
Group 2	Baseline	PRX1354 (4.9mg/kg) + benserazide (10mg/kg)
Group 3	Baseline	L-DOPA (4mg/kg) + benserazide (10mg/kg)
Group 4	Baseline	PRX1354 (9.8mg/kg) + benserazide (10mg/kg)
Group 5	Baseline	L-DOPA (8mg/kg) + benserazide (10mg/kg)
Group 6	Baseline	PRX1354 (19.6mg/kg) + benserazide (10mg/kg)

5.6 The effect of three times daily administration of PRX 1354 with benserazide compared to L-DOPA with benserazide in MPTP treated common marmosets

MPTP treated common marmosets (n=6) were removed from their home cages and their body weight recorded. Animals were then placed into locomotor activity test units. The MPTP treated marmosets were assessed over a 60-minute acclimatisation period to provide a baseline activity data. Animals were dosed with either L-DOPA (4mg/kg, p.o.) with benserazide (10mg/kg, p.o.) concomitantly or PRX 1354 at the molar equivalent concentration to L-DOPA (9.8mg/kg, p.o., respectively) plus benserazide (10mg/kg) concomitantly. Observations occurred as described in section 2.4. Each experiment was undertaken on three separate occasions with one-week washout period between sequential experiments and the overall

effects of the three test days compared to determine whether PRX 1354 produces a greater cumulative effect than seen with L-DOPA.

	Time course of activity			
	0 - 60 mins	60 – 300 mins	300 -540 mins	540 - 780 mins
Group 1	Baseline	L-DOPA (4mg/kg) + benserazide (10mg/kg)	L-DOPA (4mg/kg) + benserazide (10mg/kg)	L-DOPA (4mg/kg) + benserazide (10mg/kg)
Group 2	Baseline	PRX1354 (4.9mg/kg) + benserazide (10mg/kg)	PRX1354 (4.9mg/kg) + benserazide (10mg/kg)	PRX1354 (4.9mg/kg) + benserazide (10mg/kg)

5.7 Results

5.7.1 The effect of PRX 1354 (9.8mg/kg, molar equivalent of L-DOPA 4mg/kg) administered either alone, with benserazide (10mg/kg) or with carbidopa (12.5mg/kg)

PRX 1354 (9.8mg/kg, p.o., equivalent to L-DOPA 4.0mg/kg) in the absence of benserazide or carbidopa did not increase locomotor activity, reverse motor disability or induce dyskinesia over baseline values (Figure 5.1). In contrast administration of PRX 1354 with either benserazide (10mg/kg, p.o.) or carbidopa (12.5mg/kg) caused a significant increase in locomotor activity, a reversal of motor disability and induced dyskinesia (Figure 5.1B, D & F). Whilst PRX 1354 plus carbidopa did not have a statistically significant difference in dyskinesia expression compared to PRX1354 alone (figure 5.1F) it was still present.

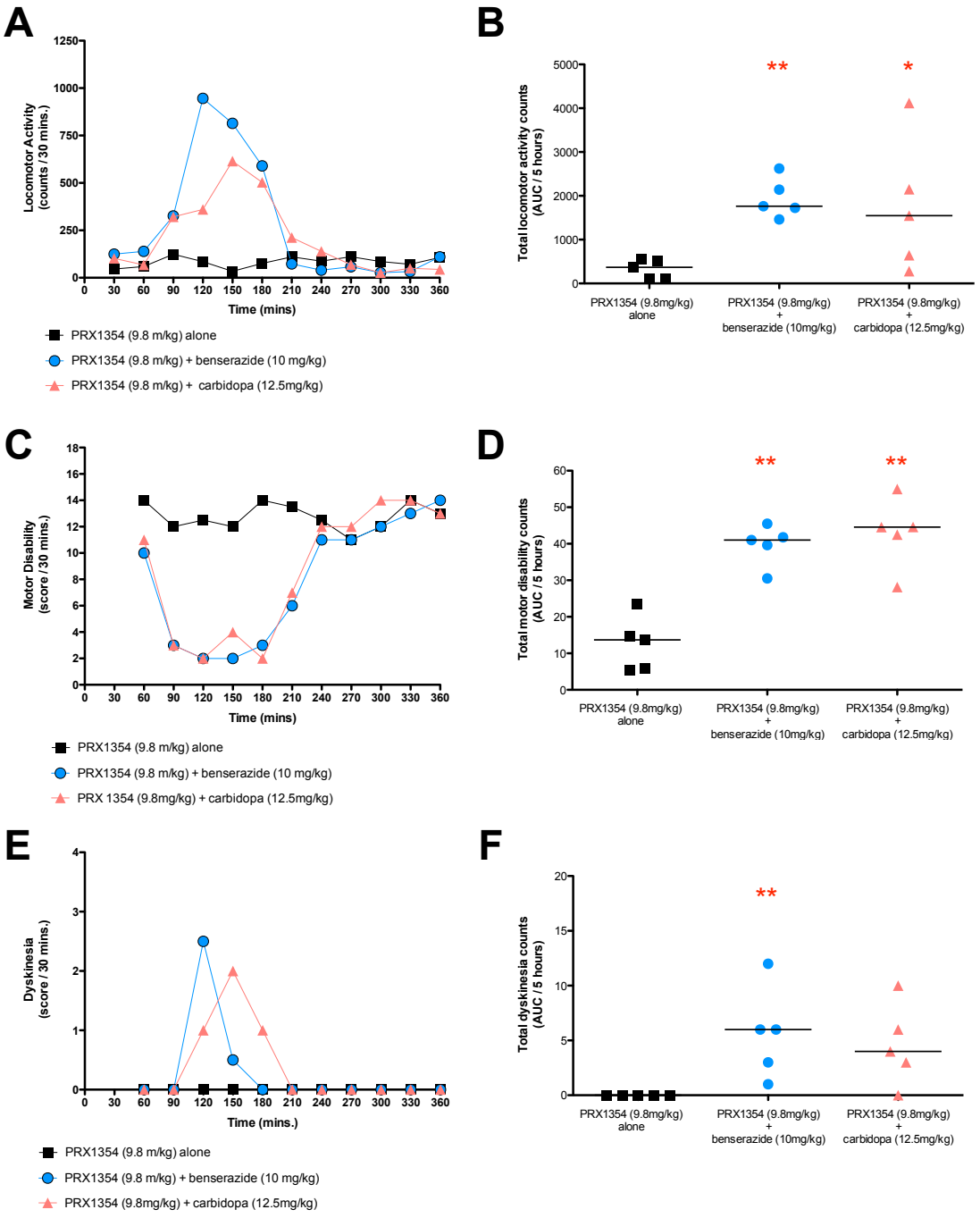

A
Locomotor Activity (counts / 30 mins.)
Time (mins)
PRX1354 (9.8 m/kg) alone
PRX1354 (9.8 m/kg) + benserazide (10 mg/kg)
PRX1354 (9.8 m/kg) + carbidopa (12.5mg/kg)
B
Total locomotor activity counts (AUC / 5 hours)
PRX1354 (9.8mg/kg) alone
PRX1354 (9.8mg/kg) + benserazide (10mg/kg)
PRX1354 (9.8mg/kg) + carbidopa (12.5mg/kg)
C
Motor Disability (score / 30 mins.)
Time (mins)
PRX1354 (9.8 m/kg) alone
PRX1354 (9.8 m/kg) + benserazide (10 mg/kg)
PRX 1354 (9.8mg/kg) + carbidopa (12.5mg/kg)
D
Total motor disability counts (AUC / 5 hours)
PRX1354 (9.8mg/kg) alone
PRX1354 (9.8mg/kg) + benserazide (10mg/kg)
PRX1354 (9.8mg/kg) + carbidopa (12.5mg/kg)
E
Dyskinesia (score / 30 mins.)
Time (mins.)
PRX1354 (9.8 m/kg) alone
PRX1354 (9.8 m/kg) + benserazide (10 mg/kg)
PRX1354 (9.8mg/kg) + carbidopa (12.5mg/kg)
F
Total dyskinesia counts (AUC / 5 hours)
PRX1354 (9.8mg/kg) alone
PRX1354 (9.8mg/kg) + benserazide (10mg/kg)
PRX1354 (9.8mg/kg) + carbidopa (12.5mg/kg)

Figure 5. 1 The effect of PRX 1354 (9.8mg/kg, molar equivalent of L-DOPA 4mg/kg) administered either alone, with benserazide (10mg/kg) or with carbidopa (12.5mg/kg)

MPTP treated marmosets (n=5) were placed into test cages at t = 0 minutes and baseline recorded. At 60 minutes, animals were treated with PRX 1354 (9.8mg/kg, p.o.) alone or with either without benserazide (10mg/kg, p.o.) or carbidopa (12.5mg/kg) and observed for a further 5 hours. Data are expressed as median time courses for locomotor activity (a) motor disability (c) and dyskinesia (e). Totals area under the curve (AUC) for locomotor activity (b), motor disability (d) and dyskinesia (f) are shown as individual values for animals with the median value indicated by the horizontal line. * $P < 0.05$ and ** $P < 0.01$ compared to PRX 1354 (9.8mg/kg) alone. Data analysed as one way-ANOVA followed by Dunnett's test.

5.7.2 Comparison of the dose-related effect of PRX 1354 administered with benserazide (10mg/kg) compared to molar equivalent doses of L-DOPA (2, 4 and 8mg/kg) plus benserazide (10mg/kg) in MPTP treated common marmosets

5.7.2.1 Locomotor activity

Both PRX 1354 and L-DOPA in combination with benserazide produced increases in locomotor activity (figure 5.2A). There was no significant difference in the locomotor activity response between L-DOPA and PRX 1354. Total locomotor activity (B) and locomotor activity 'on-time' (C) dose response curves show a significant dose-related effect on locomotor activity despite there being no significance between L-DOPA and PRX 1354.

5.7.3.2 Motor disability

Both PRX 1354 and L-DOPA with benserazide reversed motor disability. There was a significant dose related difference in the reversal of motor disability produced by PRX 1354 and L-DOPA (Figure 5.3B). Total motor disability reversal (B) and motor disability 'on-time' (C) dose response curves show a significant dose-related effect. However, for motor disability 'on-time' and total motor disability reversal there was a significant difference between L-DOPA and PRX 1354.

5.7.3.3 Dyskinesia

Both PRX 1354 and L-DOPA with benserazide induced dyskinesia expression (figure 5.4A). There was a significant dose related difference in dyskinesia expression produced by PRX 1354 and L-DOPA (Figure 5.4B). Total dyskinesia scores (5.4B) and dyskinesia 'on-time' (5.4C) dose response curves show a significant dose-related effect. There was no overall difference in dyskinesia expression between L-DOPA and PRX 1354.

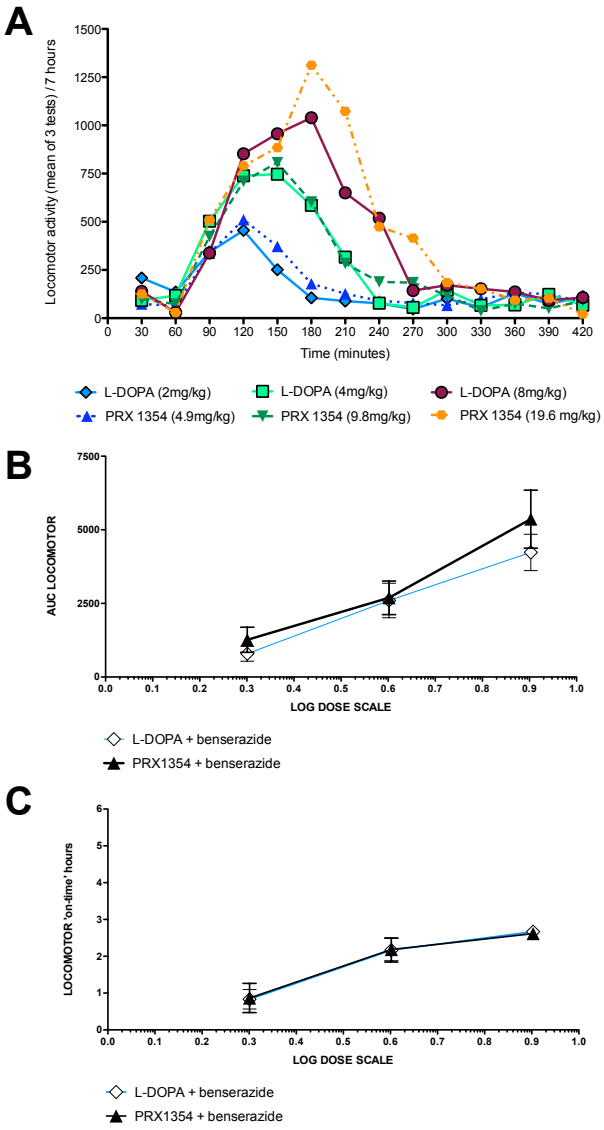


Figure 5. 2 Comparison of the dose-related effect on locomotor activity of PRX 1354 administered with benserazide (10mg/kg) compared to molar equivalent doses of L-DOPA (2, 4 & 8mg/kg) plus benserazide (10mg/kg) in MPTP treated common marmosets

MPTP treated marmosets (n=6) were placed into test cages at t= 0 minutes and baseline locomotor activity was recorded. At 60 minutes, animals were treated with L-DOPA (2, 4 or 8mg/kg, p.o.) with benserazide (10mg/kg, p.o.) or PRX 1354 (4.9, 9.8 or 19.6mg/kg, molar equivalent to L-DOPA, p.o.) with benserazide (10mg/kg, p.o.) and were observed for a further 6 hours. Animals were given at least 3 days washout between different treatments. Data are expressed as median time course for the average of 3 test days per treatment group. Time course (A), total area under the curve plotted as log dose scale (AUC) (B), 'on-time' plotted as log-dose scale (C). Log-dose data was analysed by TWO way ANOVA followed by post-hoc test.

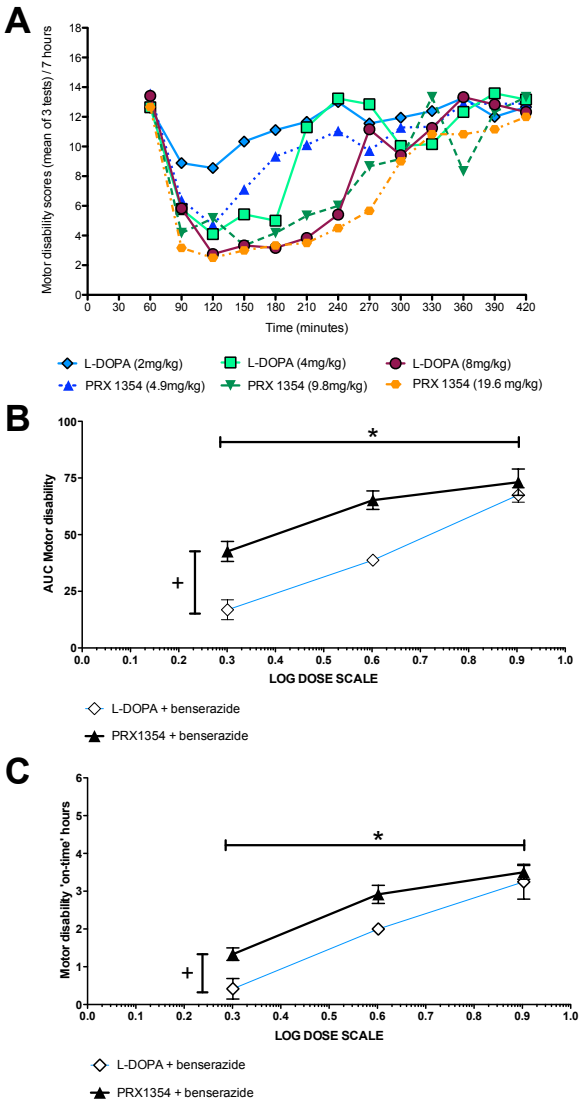


Figure 5. 3 Comparison of the dose-related effect on motor disability reversal of PRX 1354 administered with benserazide (10mg/kg) compared to molar equivalent doses of L-DOPA (2, 4 & 8mg/kg) plus benserazide in MPTP treated common marmosets

MPTP treated marmosets (n=6) were placed into test cages at t= 0 minutes and baseline motor disability was recorded. At 60 minutes, animals were treated with L-DOPA (2, 4 or 8mg/kg, p.o.) with benserazide (10mg/kg, p.o.) or PRX 1354 (4.9, 9.8 or 19.6mg/kg, molar equivalent to L-DOPA, p.o.) with benserazide (10mg/kg, p.o.) and were observed for a further 6 hours. Animals were given at least 3 days washout between different treatments. Data are expressed as median time course for the average of 3 test days per treatment group. Time course (A), total area under the curve plotted as log dose scale (B), 'on-time' plotted as log-dose scale (C). Log-dose data was analysed by TWO way ANOVA followed by post-hoc test. * $P < 0.05$ significant difference between doses (dose response) and + $P < 0.05$ significant difference between PRX 1354 and L-DOPA.

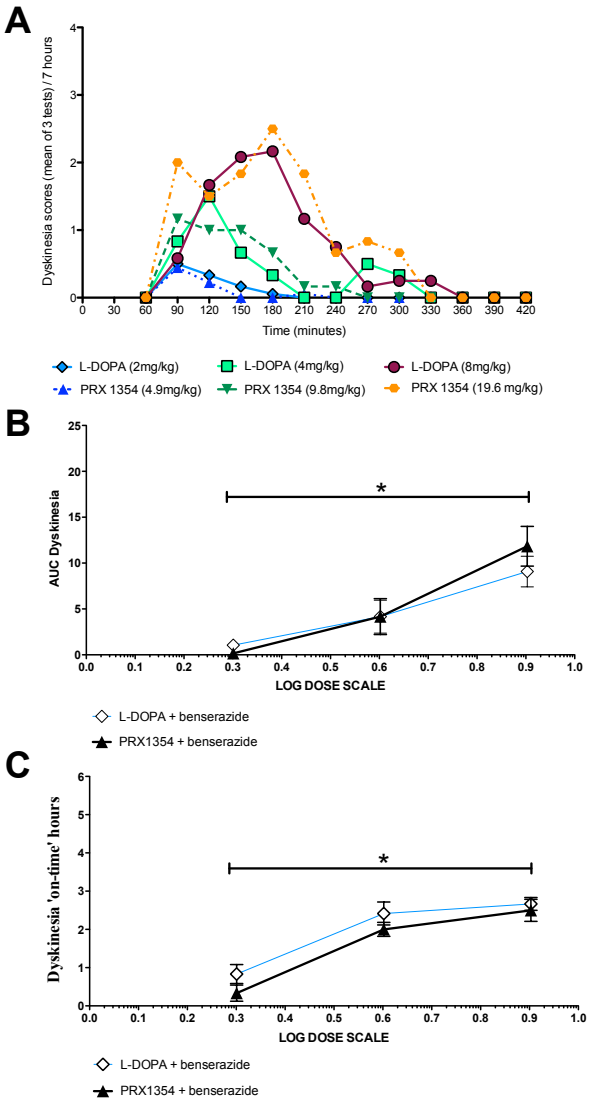


Figure 5. 4 Comparison of the dose-related effect on dyskinesia expression of PRX 1354 administered with benserazide (10mg/kg) compared to molar equivalent doses of L-DOPA (2, 4 & 8mg/kg) plus benserazide in MPTP treated common marmosets

MPTP treated marmosets (n=6) were placed into test cages at t= 0 minutes and baseline dyskinesia scores were recorded. At 60 minutes, animals were treated with L-DOPA (2, 4 or 8mg/kg, p.o.) with benserazide (10mg/kg, p.o.) or PRX 1354 (4.9, 9.8 or 19.6mg/kg, molar equivalent to L-DOPA, p.o.) with benserazide (10mg/kg, p.o.) and were observed for a further 6 hours. Animals were given at least 3 days washout between different treatments. Data are expressed as median time course for the average of 3 test days per treatment group. Time course (A), total area under the curve plotted as log dose scale (B), 'on-time' plotted as log-dose scale (C). Log-dose data was analysed by TWO way ANOVA followed by post-hoc test. * $P < 0.05$ significant difference between doses (dose response).

5.7.4 Comparison of the effect of PRX 1354 with benserazide administration three times daily (t.i.d) compared to L-DOPA with benserazide administered three times daily (t.i.d) in MPTP treated common marmosets

5.7.5.1 Locomotor activity

Both PRX 1354 (9.6mg/kg three times daily, molar equivalent to L-DOPA 4.0mg/kg, p.o.) with benserazide (10mg/kg, p.o.) and L-DOPA (4.0mg/kg, p.o., three times daily) with benserazide increased locomotor activity in each of the three experiments making up this investigation (figure 5.5A and 5.5B).

There was no significant difference in locomotor activity produced by L-DOPA t.i.d compared to PRX 1354 t.i.d daily for total locomotor activity, locomotor activity 'on-time', peak locomotor activity and the coefficient of variation for locomotor activity.

5.7.5.2 Motor disability

The administration of PRX 1354 (9.6mg/kg three times daily, molar equivalent to L-DOPA 4.0mg/kg, p.o.) t.i.d, improved total motor disability compared to L-DOPA (4.0mg/kg, p.o.) t.i.d (Figure 5.6B). PRX 1354 significantly improved motor disability 'on-time' compared to L-DOPA (figure 5.6B). There was no significant difference in peak reversal of motor disability or coefficient of variation between L-DOPA and PRX 1354.

5.7.5.3 Dyskinesia

Both PRX 1354 (9.8mg/kg three times daily, molar equivalent to L-DOPA 4.0mg/kg, p.o.) with benserazide (10mg/kg, p.o.) and L-DOPA (4.0mg/kg three times daily, p.o.) with benserazide induced dyskinesia. There was no significant difference in dyskinesia expression between L-DOPA t.i.d and PRX 1354 t.i.d treatments for total, peak or on-time dyskinesia.

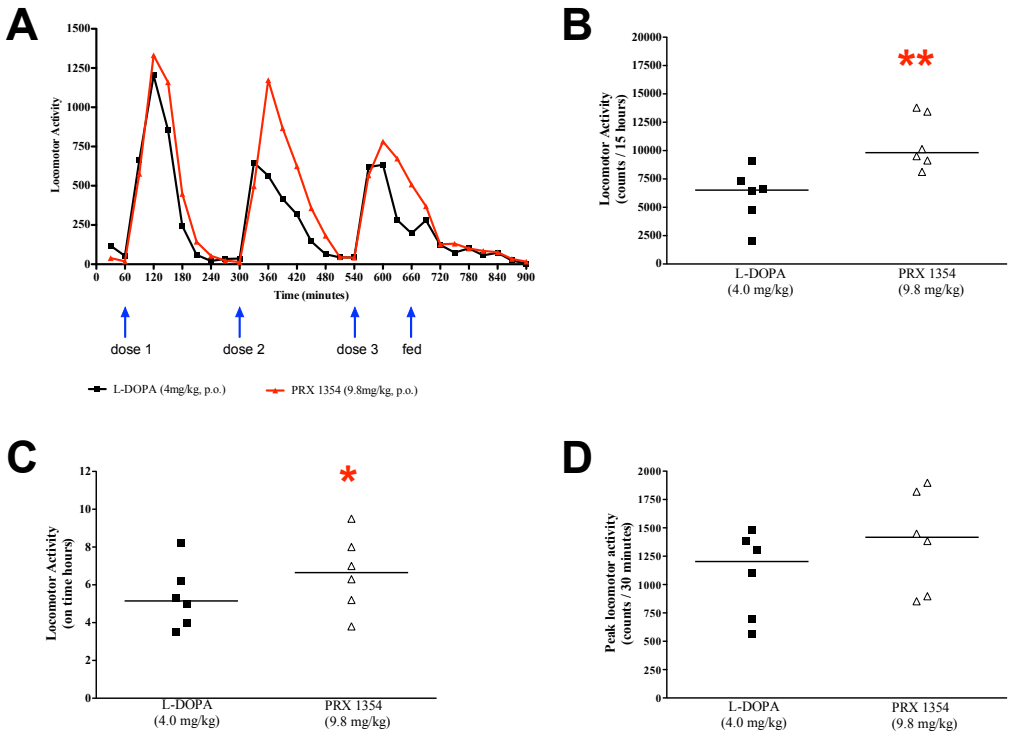


Figure 5. 5 the effect of PRX 1354 (9.8mg/kg) T.I.D compared to L-DOPA (4mg/kg) T.I.D on locomotor activity timecourse (A), total locomotor activity (B), locomotor activity 'on-time' (C) and peak locomotor activity (D) in the MPTP treated common marmoset

MPTP treated marmosets (n=6) were placed into test cages at t = 0 minutes and baseline locomotor activity was recorded for 60 minutes. At t = 60, 300 and 540 minutes animals were treated with L-DOPA (4mg/kg, p.o.) with benserazide (10mg/kg, p.o.) or PRX 1354 (9.8mg/kg, molar equivalent to L-DOPA, p.o.) with benserazide (10mg/kg, p.o.). Animals were given at least 3 days washout between different treatments. Data are expressed as medians for a mean value taken over three test days per treatment group. Time course data (a) shown as median values. Blue upward arrows represent time of drug administration and the green upwards arrow represents when food was placed into test cages. Total area under the curve (AUC), 'on-time' and peak activity are shown (b, c and d respectively) as individual values for animals with the median value indicated by the horizontal line. There was significant difference between L-DOPA and PRX 1354 for total locomotor activity AUC and on-time but not peak activity. $P < 0.05$ compared to L-DOPA (4mg/kg, p.o.) with benserazide (10mg/kg, p.o.), paired t-test on transformed data, $y=\sqrt{y}$.

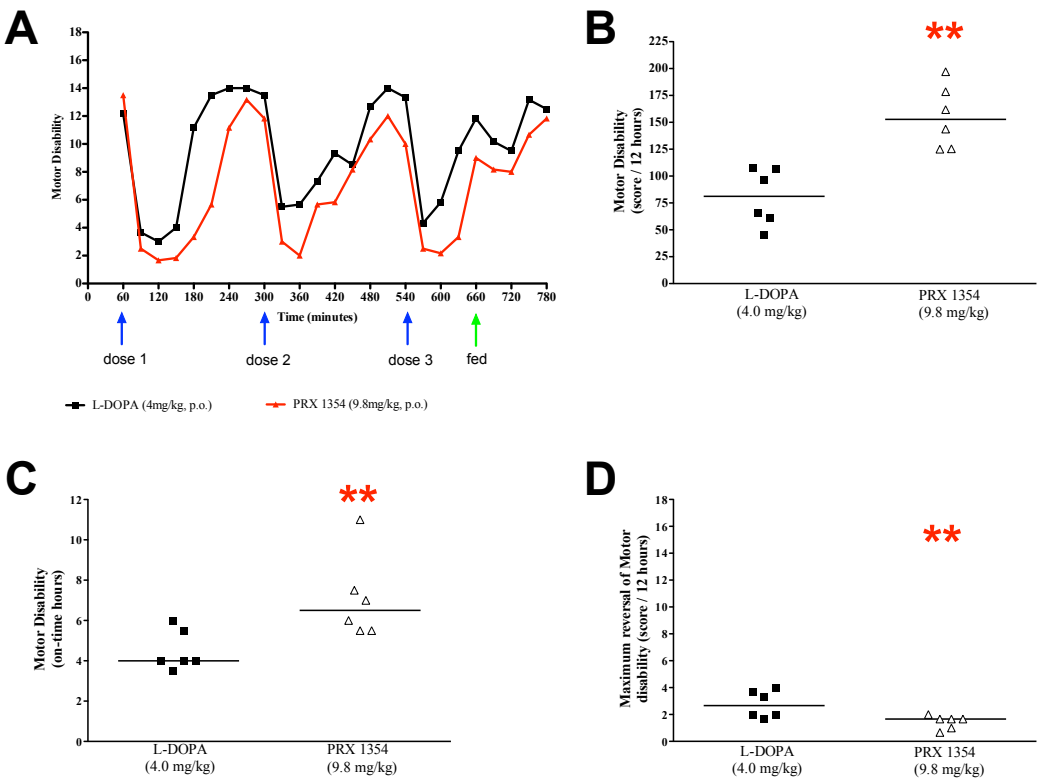


Figure 5. 6 The effect of PRX 1354 (9.8mg/kg) T.I.D compared to L-DOPA (4mg/kg) T.I.D on motor disability timecourse (A), total motor disability (B), motor disability 'on-time' (C) and peak motor disability reversal (D) in MPTP treated common marmosets

MPTP treated marmosets (n=6) were placed into test cages at t = 0 minutes and baseline motor disability score was recorded for 60 minutes. At t = 60, 300 and 540 minutes animals were treated with L-DOPA (4mg/kg, p.o.) with benserazide (10mg/kg, p.o.) or PRX 1354 (4mg/kg, molar equivalent to L-DOPA, p.o.) with benserazide (10mg/kg, p.o.). Animals were given at least 3 days washout between different treatments. Data are expressed as medians for a mean value taken over three test days per treatment group. Time course data (a) shown as median values. Blue upward arrows represent time of drug administration and the green upwards arrow represents when food was placed into test cages. Total area under the curve (AUC), 'on-time' and peak motor disability reversal are shown (b, c and d respectively) as individual values for animals with the median value indicated by the horizontal line. There was a significant difference between L-DOPA and PRX 1354 for total motor disability (AUC) (B), motor disability 'on-time' (C) and peak reversal of motor disability. ** $P < 0.01$ compared to L-DOPA (4mg/kg, p.o.) with benserazide (10mg/kg, p.o.) t.i.d, paired t-test on transformed data, $y=\sqrt{y}$.

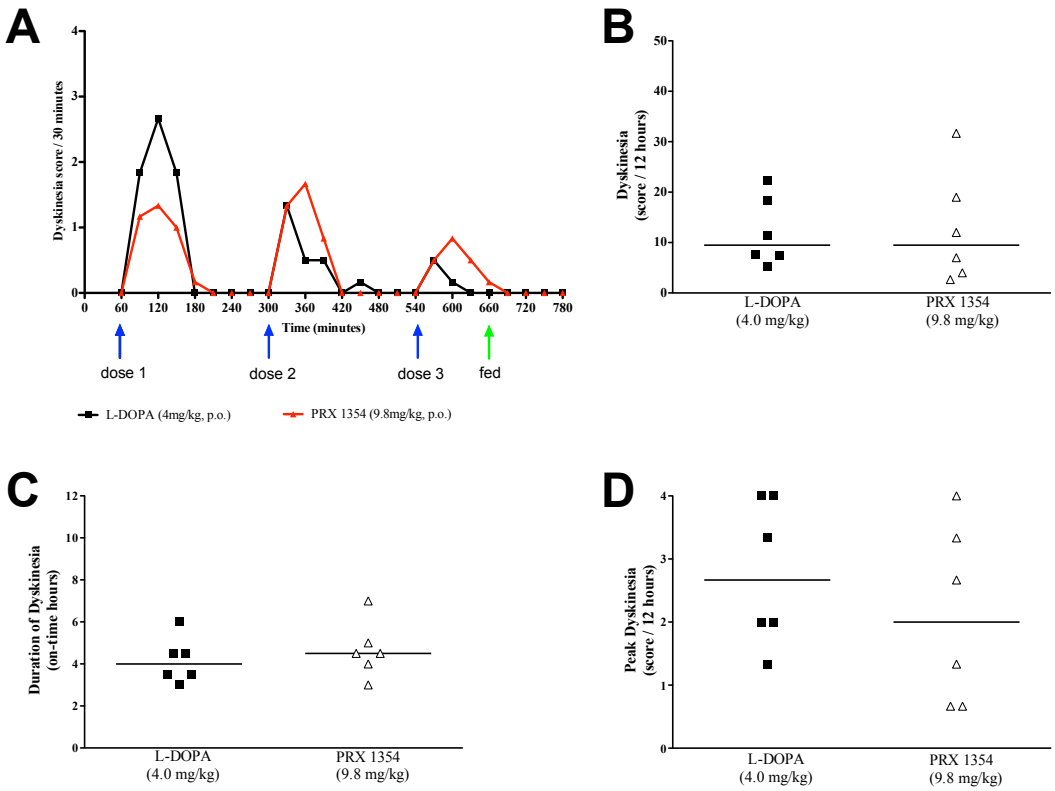


Figure 5. 7 The effect of PRX 1354 (9.8mg/kg) T.I.D compared to L-DOPA (4mg/kg) T.I.D on dyskinesia timecourse (A), total dyskinesia (B), dyskinesia 'on-time' (C) and peak dyskinesia (D) in the MPTP treated common marmoset

MPTP treated marmosets (n=6) were placed into test cages at t = 0 minutes and baseline dyskinesia score was recorded for 60 minutes. At t = 60, 300 and 540 minutes animals were treated with L-DOPA (4mg/kg, p.o.) with benserazide (10mg/kg, p.o.) or PRX 1354 (4mg/kg, molar equivalent to L-DOPA, p.o.) with benserazide (10mg/kg, p.o.). Animals were given at least 3 days washout between different treatments. Data are expressed as medians for a mean value taken over three test days per treatment group. Time course data (a) shown as median values. Blue upward arrows represent time of drug administration and the green upwards arrow represents when food was placed into test cages. Total area under the curve (AUC), 'on-time' and peak dyskinesia score are shown (b, c and d respectively) as individual values for animals with the median value indicated by the horizontal line. There was no significant difference between L-DOPA and PRX 1354 for all dyskinesia parameters. $P > 0.05$ compared to L-DOPA (4mg/kg, p.o.) with benserazide (10mg/kg, p.o.) T.I.D, paired t-test on transformed data, $y=\sqrt{y}$.

5.8 Discussion

The hypothesis driving the experiments described in this chapter was whether we could improve the efficacy of L-DOPA and reduce the expression of dyskinesia in animal models of Parkinson's disease by altering the chemical formulation of L-DOPA to a dipeptide pro-drug of L-DOPA.

This chapter revolves around the hypothesis that by improving the pharmacokinetic profile of L-DOPA via a pro-drug strategy, we can improve the L-DOPA induced response in the MPTP-treated common marmoset. The design of the prodrug of L-DOPA was developed in order to overcome some of the shortcomings of standard L-DOPA treatment. PRX 1354 was designed to be better absorbed, to be resistant to metabolic degradation, to provide more continuous availability of L-DOPA and potentially to enter the brain more efficiently and then be transformed into L-DOPA and dopamine in the striatum as assessed by its actions in animal models of Parkinson's disease. Whilst these results suggest some minor advantages of the molecule in certain Parkinson's disease patients / circumstances (Zhou et al. 2010), overall they did not show an improved L-DOPA induced response when compared to standard L-DOPA treatment.

The first investigation assessed whether PRX 1354 will exert an anti-parkinsonian effect without concomitant peripheral dopa-decarboxylase inhibitor treatment. When PRX 1354 was administered alone, there was no apparent effect on any of the parameters assessed in the MPTP treated

marmosets, which had been selected for the study based on prior responsiveness to L-DOPA treatment. The failure to see effects on motor function could be interpreted as using too low a dose, lack of absorption from the gastro-intestinal tract or degradation of PRX 1354 by enzyme systems that do not release L-DOPA from the pro-drug. However, when PRX 1354 was administered with either carbidopa or benserazide there was an L-DOPA-like induced effect, which was almost identical to standard L-DOPA treatment. In this respect it is unlikely that removal of R1 and R2 from the catechol, moiety might make the molecule prone to extensive metabolism by COMT. This would indicate that the majority of the peripheral metabolism of PRX 1354 is by DDC and not by COMT (Espinoza et al. 2012).

R3, which was a methyl-based group, was designed as an ester linkage to be rapidly removed by esterases after the drug was absorbed. Upon cleavage of R3, methyldopa would have been the by-product of this enzymatic reaction. If this did not occur, then the molecule would not be a substrate for DDC and no L-DOPA would be liberated from PRX 1354 and there would be no effect. In reality this seems unlikely as such ester linkages are readily cleaved (Sozio et al. 2012) and this leads to the most likely possibility that PRX 1354 is converted to L-DOPA in the periphery (and not in the brain) and that peripheral DDC degrades the L-DOPA formed in exactly the same manner as occurs after the administration of standard L-DOPA itself (Kaakkola. 2010) which is why the DDCI helps.

In previous studies (chapters 3 and 4), the L-DOPA response was evaluated when used with either carbidopa or benserazide in the MPTP model. Those studies showed that at the doses employed, there was no significant difference between the two DDCIs in the MPTP model. In this chapter we assessed the effects of PRX 1354 administration in conjunction with carbidopa or benserazide to assess whether any behavioural differences, which could indicate different modes of action of PRX1354 compared to L-DOPA, could be assessed.

As expected the administration of PRX 1354 with either carbidopa or benserazide led to a reversal of motor deficits and expression of dyskinesia. This clearly indicates that peripheral formation of L-DOPA from PRX 1354 occurs and shows removal of R1 and R2, cleavage of the ester linkage, R3 (methyl group based moiety which is demethylated) to the carboxylic acid group and degradation of the amide bond of R4 (second L-DOPA molecule) to the amino group of the parent L-DOPA molecule. This experiment also showed that there was no difference between the two decarboxylase inhibitors irrespective of their somewhat different mechanisms of action (Da Prada et al., 1987). So the question becomes whether PRX 1354 produces L-DOPA and maintains its levels in plasma and brain in a manner that differs from that occurring after administration of L-DOPA itself (Lee et al. 2013).

L-DOPA is clinically administered at varying doses dependent on disease stage, complications and on-set of disease. Here we set out to evaluate the

dose response relationship of PRX 1354 compared to molar equivalents of L-DOPA to investigate whether we could reverse motor deficits in MPTP treated common marmosets and reduce dyskinesia.

The difference between PRX 1354 and L-DOPA were explored using three dosage levels designated as low, medium and high and investigated by using dose response analytics. The results showed no marked difference between the two drugs in terms of locomotor activity and dyskinesia suggesting that PRX 1354 is rapidly converted in the intestinal tract to an equivalent amount of L-DOPA following oral administration. However, to our surprise, PRX 1354 was significantly different to L-DOPA in terms of its effects on motor disability reversal. PRX 1354 is made up from two L-DOPA molecules joined via a peptide bond, which we would expect upon cleavage to result in twice as much L-DOPA and dopamine production which should have resulted in a more noticeable difference compared to L-DOPA especially at the lower doses. This in future experiments can be measured directly via blood sampling and HPLC analysis to assess whether two L-DOPA molecules are released from the parent and then via dialysis assess whether twice as much L-DOPA is found in the extracellular fluid. In addition LCMS analysis would allow detection of remaining L-DOPA-containing moieties, which were unable to release the L-DOPA into tissue. Whilst the response of PRX 1354 is not double that of L-DOPA it was significantly different, implying that the theoretical mechanism for which PRX 1354 was designed is not having the desired effect of being cleaved to release two molecules of L-DOPA.

All of these points may indicate that the design of PRX 1354 to produce some L-DOPA rapidly and some more slowly through phased degradation works to a degree. These results are in line with Felix and colleagues who examined various peptide derivatives of L-DOPA. Although they assessed the effects in reserpinised mice, they also found no significant and improved efficacy over standard L-DOPA (Di Stefano et al. 2008).

PRX 1354 seemed to suggest a dose dependent improvement in efficacy in relation to motor disability reversal which we explored further to examine if three times daily administration of PRX 1354 produces a cumulative improvement in locomotor activity, motor disability and dyskinesia expression compared to three times daily administration of the molar equivalent of L-DOPA.

The suggestion that PRX 1354 might have a modest advantage over L-DOPA at moderate doses led to the design of a study to test the effects of three times daily administration on one day. PRX 1354 showed significant improvement in locomotor activity (figure 5.5) parameters (total AUC and 'on-time') and motor disability (figure 5.6) parameters (total AUC, 'on-time' and peak reversal of disability score) but not on dyskinesia (figure 5.7). The design of PRX 1354 to produce some L-DOPA over a longer time course might not be apparent in the short term but might be more readily noticeable over time and with accumulation of the fragment of PRX 1354 derived from R4 that degrades to L-DOPA. Perhaps a clue that this occurs is seen by looking at the effect of the last of the three doses administered

on each test day that show a longer duration of effect on motor function than seen with L-DOPA (figure 5.6A). Also of interest is the fact that between doses on any treatment day, animals treated with PRX 1354 did not return to baseline levels of motor disability suggesting some residual drug effect that was not seen with L-DOPA itself.

Whilst improved reversal of motor disability is a key component of any improved L-DOPA formulation, the inability to reduce dyskinesia still makes PRX 1354 an unattractive clinical candidate. Whilst it is essential that new drug treatments for Parkinson's disease, induce locomotor activity in the MPTP treated common marmoset, it is unknown how this increase in locomotor activity translates into clinically meaningful patient responses. Dyskinesia is however, a treatment-limiting factor for Parkinson's disease patients, and new L-DOPA formulations that don't help reduce the expression or incidence of dyskinesia will have a limited value. These studies which utilised an L-DOPA dose of 4mg/kg, are more reflective of treating early / newly diagnosed Parkinson's disease which utilises lower doses of L-DOPA. The clinical scenario is different. With increasing dose failures, erratic absorption issues and increased incidence of dyskinesia, the doses of L-DOPA rapidly increase and therefore the beneficial effects of PRX 1354 would be masked by its inability to reduce dyskinesia (Potts et al. 2013).

The final conclusion of PRX 1354 is that it offers no significant advantage over current L-DOPA doses as described in these studies. As detailed in the introduction to this thesis, many attempts at designing prodrug derivatives

of L-DOPA have been made but none have so far succeeded. Simple ester derivatives ranging from ethyl esters through to pivatoyl esters have not increased the efficacy or duration of effect of the derived L-DOPA (Di Stefano et al. 2008) although they have markedly improved solubility. Indeed L-DOPA methyl ester is marketed (Sirio®**)** for the treatment of Parkinson's disease merely based on its increased water solubility and rapid absorption leading to a quicker onset of therapeutic response (Juncos et al. 1987). L-DOPA ethyl ester was taken into trials but showed no superiority over immediate release L-DOPA. The value of the peptide pro-drug formulations have shown that they can potentially offer superior ADME profiles to L-DOPA based on their absorption by a peptide transporter mechanism found extensively along the length of the gastro-intestinal tract (Christiaans et al. 1996). Early clinical studies have suggested that this does translate into improved clinical efficacy but large-scale clinical investigations have yet to be undertaken. Due to changing drug approval guidelines, the difficulty of approving new L-DOPA based drugs, holds little hope due to their side effect profiles and cost effectiveness compared to current generic L-DOPA. In recent years, the pharmaceutical company IMPAX laboratories had developed a new L-DOPA formulation but due to the side effects and limited improvement over current standard L-DOPA treatment, it has failed to be approved by Food and Drug Administration (FDA) and European Medicines Agency (EMA) (ClinicalTrials.gov Identifier: NCT01411137).

In conclusion, PRX 1354 offers little advantage over the administration of L-DOPA itself. Further attempts at prodrug formation may eventually overcome the erratic absorption, metabolism and effect of L-DOPA and lengthen its duration of action. However, the indications so far are that this will not be easy to achieve. Another treatment option, which can be utilised in the clinical setting, is L-DOPA sparing regimens, which combine potent dopamine agonists with low dose L-DOPA to improve motor disability whilst reducing the onset, severity and expression of dyskinesia. One such dopamine agonist is pramipexole and the following chapter investigates whether low dose L-DOPA in combination with pramipexole in drug naive MPTP-treated common marmosets can reduce the expression of dyskinesia and delay its onset.

Chapter 6

Investigation into the effect of L-DOPA combination therapy with the dopamine agonist pramipexole to reverse motor disability and reduce the risk of dyskinesia

6. Investigation into the effect of L-DOPA combination therapy with the dopamine agonist pramipexole to reverse motor disability and reduce the risk of dyskinesia

6.1 Introduction

As described in the previous Chapters, the effects on motor disability reversal and dyskinesia may be optimised by the use of DDCIs or by developing a prodrug approach to therapy. However, none of these approaches have significantly reduced the incidence of dyskinesia (Olanow and Schapira. 2013). In recent years, dopamine agonists have become more commonly used alternative approaches for treating early Parkinson's disease as they can control motor symptoms but delay the appearance of motor complications, with a notable reduction in severity of dyskinesia (Hobson et al. 1999; Schapira 2005). This has been attributed to their longer duration of effect compared to L-DOPA providing a more physiological and continuous stimulation of striatal dopamine receptors (Obeso et al. 1987; Stocchi et al. 2001). However, dopamine agonists do not possess the efficacy of L-DOPA and sooner or later L-DOPA needs to be introduced into treatment to provide adequate control of motor disability (Olanow and Schapira. 2013). Dopamine agonists are also used as an adjunct therapy to L-DOPA in the later stages of Parkinson's disease in individuals who experience motor fluctuations in the form of a shortening of the duration of effect of each L-dopa dose ('wearing off') and unpredictable swings in motor response ('on off') (Jankovic 2005; Stocchi

et al. 2008). Dopamine agonists are also used in the early stages of Parkinson's disease where L-DOPA is added to existing DA agonist treatment. Dopamine agonists provide a more prolonged reversal of motor deficits through their longer duration of action (Hobson et al. 1999; Montastruc et al. 1999; Rascol et al. 2000b; Hubble 2002; Oertel et al. 2006; Jackson et al. 2007). However, in those individuals who already exhibit dyskinesia in response to L-dopa, the addition of a long acting dopamine agonist can lead to a worsening of the duration of involuntary movements that may become treatment limiting (Diamond et al. 1984; Bonuccelli et al. 2002). Thus, it is unclear whether DA agonists can improve L-DOPA induced fluctuations.

It is suggested that dyskinesia seen in mid-to-late stage Parkinson's disease can be reduced by the introduction of more continuous dopaminergic therapy. This has been shown after the use of subcutaneous infusions of apomorphine and more recently after the continuous intra-jejunal infusion of L-DOPA (DuoDopa) (Albani et al. 1992; Zaleska et al. 1999; Stocchi et al. 2001; Oertel et al. 2006; Rudzinska et al. 2007).

In studies in MPTP-treated primates, we have previously demonstrated that switching from repeated L-dopa treatment that has induced dyskinesia to monotherapy with a dopamine agonist, maintains efficacy but leads to an immediate reduction in dyskinesia intensity (Smith et al. 2006; Jackson et al. 2007; Stockwell et al. 2008). Perhaps, most importantly we showed that an agonist dominant combination of ropinirole and L-DOPA resulted in less

dyskinesia than an L-DOPA dominant combination of the two drugs (Maratos et al. 2001).

One hypothesis is that combinations of L-DOPA and dopamine agonists might be used to achieve the required control of motor symptoms while utilising the lower dyskinesia potential of dopamine agonists and their longer half life to diminish involuntary movements. However, this has not been extensively studied in man or in MPTP-treated primates but some clues already exists to suggest this might form a viable strategy (Shimazu et al. 2003).

Therefore it was hypothesised that a combination of a long-acting dopamine agonist with L-DOPA can maintain motor control (motor disability reversal) while reducing the priming for dyskinesia in MPTP-treated common marmosets, thereby increasing the therapeutic window of L-DOPA utility. For this reason, the effects of L-DOPA alone and combined with the dopamine agonist pramipexole have been studied in drug naïve MPTP-treated common marmosets.

6.2 Aims

In order to test this hypothesis, a chronic 56-day dosing study was performed in in MPTP-treated common marmosets investigating the effect of L-DOPA administration alone and in combination with pramipexole. This study had the following aims:

- To compare the ability of L-DOPA and pramipexole to prime for dyskinesia expression in drug naïve MPTP-treated common marmosets
- To determine whether low dose combination treatment with pramipexole and L-DOPA could maintain motor function with reduced dyskinesia
- To assess whether early initiation with pramipexole results in lower dyskinesia levels once L-DOPA is combined to the treatment regimen

6.3 Methods and materials

A brief overview of the materials and methods used are given here but the detailed methodology is to be found in Chapter 2.

6.3.1 Animals

Vasectomized male and female adult common marmosets (350g or above) (Harlan UK) were given two weeks to acclimatize prior to MPTP treatment. Animals were housed either in single cages or in male and female pairs. Animals were placed in holding rooms which operate on a 12 hour light / dark cycle, 50% humidity at a temperature of 25 ± 1°C. Animals had ad libutum access to Mazuri pellets and water at all times. Animals were

treated with MPTP as described in section 2.3.4 in chapter 2. Following MPTP treatment animals were allowed 8-12 weeks for motor deficits to stabilize prior to the start of the study. On day 0 baseline locomotor activity was recorded as described in chapter 2. Animals were allocated into 3 balanced groups based on their locomotor activity scores.

6.3.2 Drugs

The drugs used for the study carried out in this section are detailed below:

L-3,4-dihydroxyphenylalanine methyl-ester, (*L-DOPA; 3.125 - 12.5 mg/kg, free base; Lot No. 043K5000; Sigma Chemical Co, UK*) was dissolved in a 10% sucrose solution and administered orally in a volume of 2.0 ml/kg.

Carbidopa (12.5 mg/kg; Lot No. 103K1347; Sigma Chemical Co, UK) was administered orally as a suspension in a 10% sucrose solution in a volume of 2.0 ml/kg. Carbidopa was given as a pre-treatment 1 hour prior to the first of the twice-daily L-DOPA administrations and concomitantly with the second administration of L-DOPA.

Pramipexole HCl (0.04 - 0.3 mg/kg; Lot No. 101207; Boehringer Ingelheim, Germany) was dissolved in a 10% sucrose solution and administered orally in a volume of 2.0 ml/kg.

Domperidone (2.0 mg/kg; Lot No. 031K4611; Sigma Chemical Co, UK) was administered orally as a suspension in a 10% sucrose solution in a volume

of 2.0 ml/kg. Domperidone was administered as required to prevent vomiting/retching following administration of pramipexole. On test days domperidone was given as a pre-treatment 1 hour prior to the first of the twice-daily pramipexole treatments and in combination with the second pramipexole treatment.

6.3.3 Drug treatments

Animals were dosed according to the schedule in table 6.1. Animals had doses adjusted in between test days. Additional tests were carried out on animals during both phases but data is not included.

Drug treatment was divided into three phases:

Phase I: chronic treatment for days 0 – 35 (no drug combinations)

Phase II: chronic treatment for days 36 – 56 (L-DOPA combination therapy)

Phase III: 3 acute L-DOPA challenges (one per week)

The 3 groups of drug naïve MPTP-treated common marmosets were allocated to three treatment groups:

Group 1: (L-DOPA only)

Phase I – L-DOPA (12.5mg/kg + benserazide 10mg/kg, p.o.) b.i.d

Phase II – L-DOPA (10 – 12.5mg/kg + benserazide 10mg/kg, p.o.) b.i.d

Phase III – L-DOPA (12.5mg/kg + benserazide 10mg/kg, p.o.) b.i.d

Group 2: (pramipexole only)

Phase I – pramipexole (0.04 – 0.3mg/kg, p.o.)

Phase II – pramipexole (0.2mg/kg, p.o.) b.i.d

Phase III - L-DOPA (12.5mg/kg + benserazide 10mg/kg, p.o.) b.i.d

Group 3 (combination group)

Phase I – pramipexole (0.04 – 0.3mg/kg, p.o.) s.i.d or b.i.d

Phase II – pramipexole (0.15 – 0.2mg/kg, p.o.) s.i.d + L-DOPA (3.125-6.25mg/kg, p.o. + benserazide 10mg/kg) b.i.d

Phase III - L-DOPA (12.5mg/kg + benserazide 10mg/kg, p.o.) b.i.d

Doses of pramipexole and L-DOPA were adjusted over Phase I and Phase II to match anti-parkinsonian responses.

6.3.4 Behavioural assessment

All animals were assessed for basal motor activity, motor disability and dyskinesia prior to the start of PHASE I treatment (baseline) as described in chapter 2. Animals were then treated on a daily basis and locomotor activity, motor disability and dyskinesia was assessed on days 1, 3, 5, 7 and then once-a-week until day 35 (PHASE I), and on days 36, 38, 40, 42 and then once-a-week until day 56 (PHASE II). Acute L-DOPA challenges (PHASE III) were carried at weekly intervals following the cessation of chronic treatment.

It should be noted that it was necessary to adjust the dose regime of pramipexole during PHASE I and for the animals receiving L-DOPA plus pramipexole during PHASE II, to maintain equivalence based on locomotor activity with animals receiving L-DOPA BID, alone with a single daily dose of pramipexole being utilised from day 36. In PHASE II some animals receiving L-DOPA showed such severe dyskinesia that dose reduction became necessary. The dose of L-DOPA was reduced to 10 mg/kg in 4 animals from the group receiving L-DOPA BID, alone and to 3.125 mg/kg in 1 animal from group receiving L-DOPA BID plus pramipexole, SID. (Table 6.1: Treatment schedule).

6.3.5 Test day protocol

Animals were acclimatised to the automated activity (test) units for 60 minutes following pre-treatment with carbidopa (12.5 mg/kg) or domperidone (2.0 mg/kg). Each test unit was fitted with 8 photoelectric

beams arranged to detect floor, perch and climbing activity, interruption of a beam being automatically recorded as a single locomotor count. During this time baseline locomotor activity, motor disability and dyskinesia was determined according to established protocols (Jackson et al. 2007; Zubair et al. 2007). Pramipexole or L-DOPA treatment was then administered at time = 60 and 300 minutes, after which locomotor activity, motor disability and dyskinesia was monitored for up to 8 hours following the first administration of drug.

After 56 days of chronic drug administration (both PHASE I and II) animals were on three separate occasions at weekly intervals challenged acutely with L-DOPA (12.5mg/kg + benserazide 10mg/kg, p.o.) b.i.d at 0 and 4 hours. Animals were assessed for locomotor activity, motor disability and dyskinesia (phase III) as described in section 2.3.

6.3.6 Data and Statistical Analysis

All data was treated as non-Gaussian. Locomotor activity counts, motor disability scores and dyskinesia scores are presented as medians in time course data and median and range when assessing total locomotor activity counts, motor disability and dyskinesia and peak dyskinesia scores. Total locomotor activity counts, motor disability and dyskinesia scores were determined by area under the curve as described in chapter 2. Time course data was not analysed statistically. Differences in area under the curve for locomotor activity, motor disability and dyskinesia and peak dyskinesia

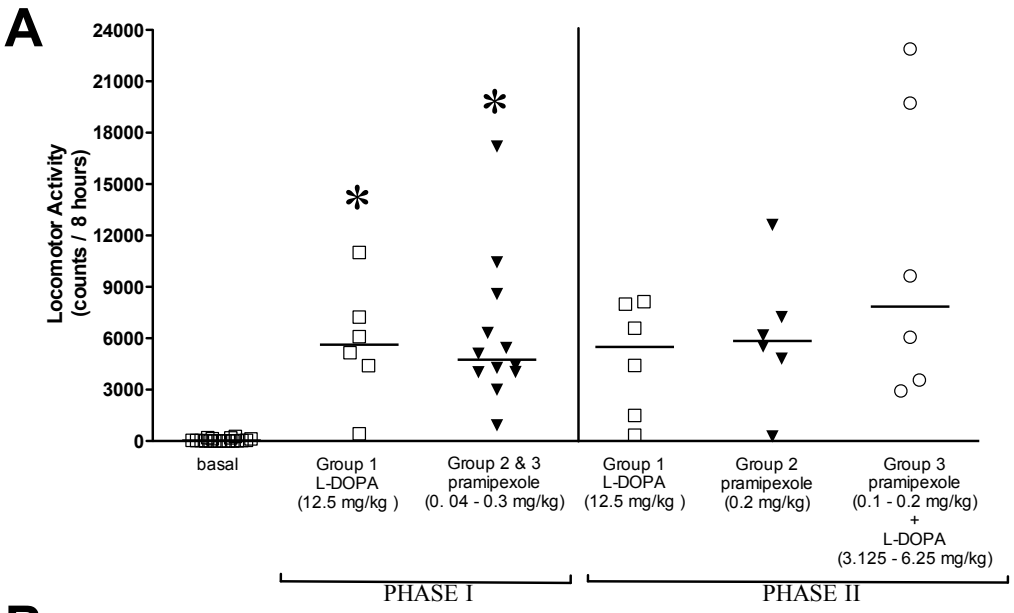
were determined by non-parametric one way ANOVA followed by a Dunnett's post-hoc test (GraphPad Prism®) or Mann Whitney as necessary. Treatment days within each group for PHASE I were compared to basal assessments (day 0) and during PHASE II each of the three treatment groups were compared to each other. A comparison between treatment groups was made using the median of the overall assessments for each phase.

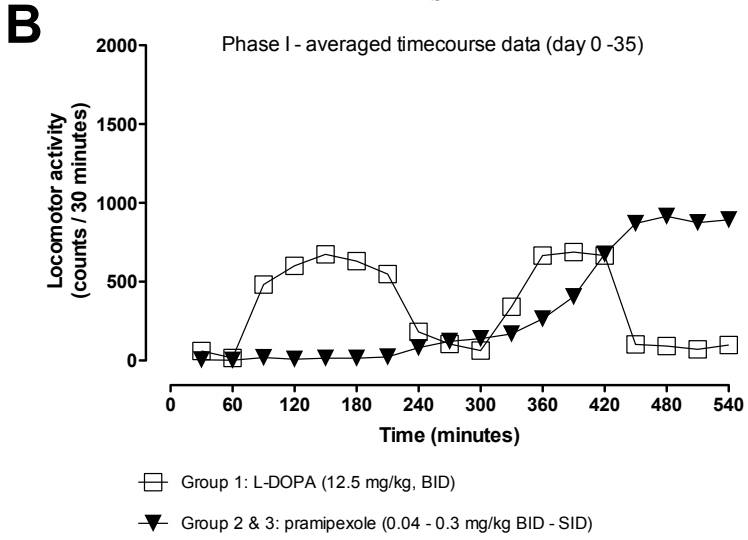
6.4 Results

6.4.1 Assessment of locomotor activity over PHASE I and II of the study

Both L-DOPA (group 1) and pramipexole (group 2&3 combined) increased locomotor activity during Phase I compared to baseline levels (figure 6.1A) with no difference in locomotor activity between the two treatment groups.

Similarly locomotor activity remained raised following L-DOPA (group 1), pramipexole alone (group 2) and L-DOPA plus pramipexole (group 3) during Phase II with no difference between treatment groups. The locomotor activity profile between L-DOPA (group 1) and pramipexole (group 2&3) was different. Group 2&3 experienced a delay in locomotor activity following administration of the 1st drug dose and activity was above baseline levels at the end of the test day(s) (figure 6.1B). This response was sustained during Phase II (figure 6.1C).


A
Locomotor Activity
(counts / 8 hours)
24000
21000
18000
15000
12000
9000
6000
3000
0
*
*
basal
Group 1
L-DOPA
(12.5 mg/kg)
Group 2 & 3
pramipexole
(0. 04 - 0.3 mg/kg)
Group 1
L-DOPA
(12.5 mg/kg)
Group 2
pramipexole
(0.2 mg/kg)
Group 3
pramipexole
(0.1 - 0.2 mg/kg)
+
L-DOPA
(3.125 - 6.25 mg/kg)
PHASE I
PHASE II


B
Phase I - averaged timecourse data (day 0 -35)
Locomotor activity
(counts / 30 minutes)
2000
1500
1000
500
0
0
60
120
180
240
300
360
420
480
540
Time (minutes)
Group 1: L-DOPA (12.5 mg/kg, BID)
Group 2 & 3: pramipexole (0.04 - 0.3 mg/kg BID - SID)

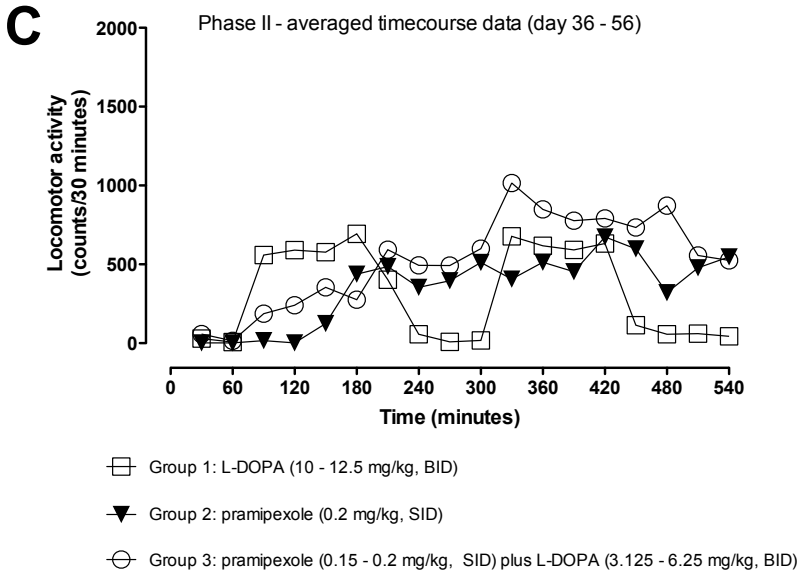

C
Phase II - averaged timecourse data (day 36 - 56)
Locomotor activity
(counts/30 minutes)
2000
1500
1000
500
0
0
60
120
180
240
300
360
420
480
540
Time (minutes)
Group 1: L-DOPA (10 - 12.5 mg/kg, BID)
Group 2: pramipexole (0.2 mg/kg, SID)
Group 3: pramipexole (0.15 - 0.2 mg/kg, SID) plus L-DOPA (3.125 - 6.25 mg/kg, BID)

Figure 6. 1 Total locomotor activity counts (A) and mean timecourses (B&C) for PHASES I and II

PHASE I: L-DOPA (12.5 mg/kg, p.o, plus carbidopa 12.5 mg/kg, p.o, BID, n=6); pramipexole (0.04 - 0.3 mg/kg, p.o, plus domperidone 2.0 mg/kg, p.o, BID – SID, n=12).

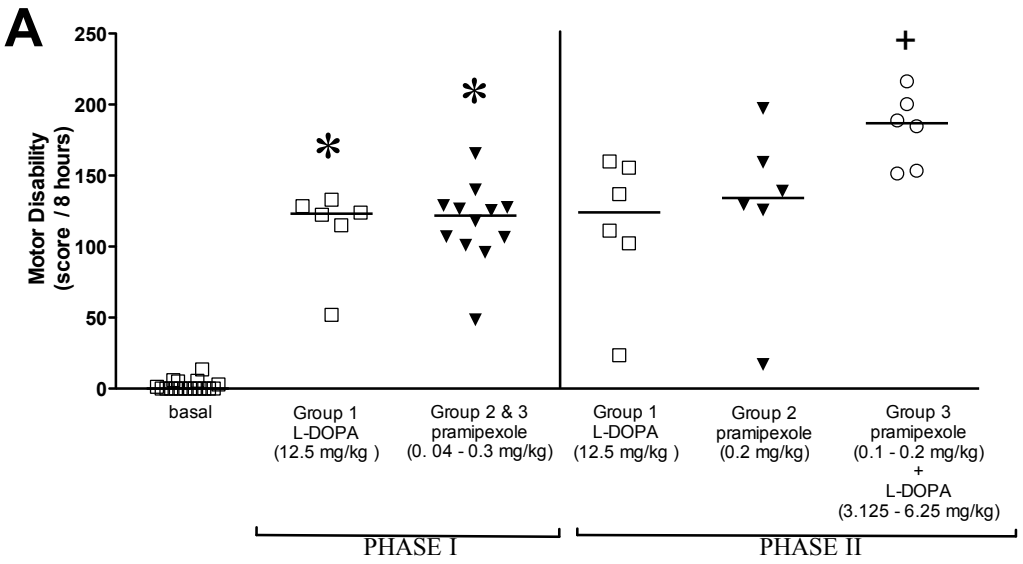
PHASE II: L-DOPA (10 - 12.5 mg/kg, p.o, plus carbidopa 12.5 mg/kg, p.o, BID, n=6); pramipexole (0.1 - 0.2 mg/kg, p.o, plus domperidone 2.0 mg/kg, p.o, SID, n=6) plus L-DOPA (3.125 – 6.25 mg/kg, p.o, plus carbidopa 12.5 mg/kg, p.o, BID); pramipexole (0.2 mg/kg, p.o, plus domperidone 2.0 mg/kg, p.o, SID, n=6).
Data (n = 6 or 12) are presented as the median and range of the locomotor activity counts of the averaged test days for PHASE I (days 7, 14, 21, 28, 35) and PHASE II (days 42, 49, 56 and 63) (A). Averaged timecourse data are presented as median values for Phase I (B) and Phase II (C). * Indicates that locomotor activity was significantly greater than basal locomotor activity for PHASE I (Kruskal Wallis $p<0.0001$, Mann Whitney $p < 0.05$).

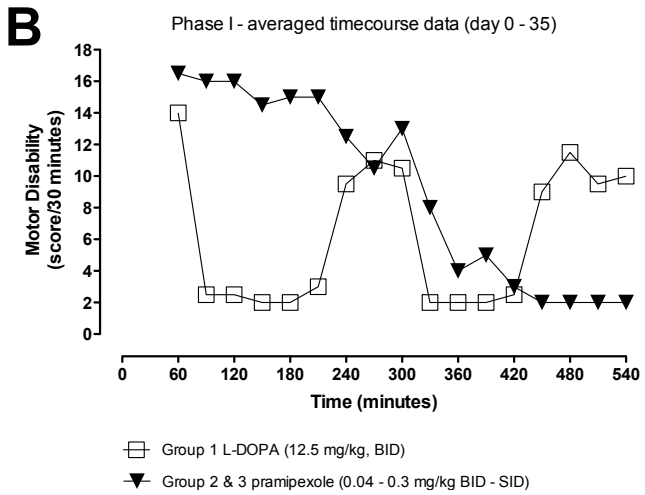
6.4.2 Assessment of motor disability over PHASE I and II of the study

During PHASE I, both groups (1 and 2/3), receiving L-DOPA or pramipexole treatments reversed motor disability significantly compared to their baseline scores prior to drug treatment (figure 6.2A).

The improved reversal of motor disability seen during PHASE I with both group 1 and 2/3 was sustained in PHASE II. During PHASE II, group 3 (L-DOPA plus pramipexole) significantly improved the reversal of motor disability compared to pramipexole alone (group 2) (Figure 6.2A).

The motor disability timecourse profile in Phase I was different between group 1 and 2/3 (figure 6.2B). The pramipexole alone group took 2-3 hours before seeing a beneficial improvement in motor function and this gradually improved and was sustained for the remainder of the test period. This response was sustained during Phase II whereby group 2 and 3 induced motor function improvement, which was greater than baseline levels even at the end of the test period (figure 6.2C).


A
Motor Disability
(score / 8 hours)
250
200
150
100
50
0
*
*
+
basal
Group 1
L-DOPA
(12.5 mg/kg)
Group 2 & 3
pramipexole
(0. 04 - 0.3 mg/kg)
Group 1
L-DOPA
(12.5 mg/kg)
Group 2
pramipexole
(0.2 mg/kg)
Group 3
pramipexole
(0.1 - 0.2 mg/kg)
+
L-DOPA
(3.125 - 6.25 mg/kg)
PHASE I
PHASE II


B
Phase I - averaged timecourse data (day 0 - 35)
Motor Disability
(score/30 minutes)
18
16
14
12
10
8
6
4
2
0
0
60
120
180
240
300
360
420
480
540
Time (minutes)
Group 1 L-DOPA (12.5 mg/kg, BID)
Group 2 & 3 pramipexole (0.04 - 0.3 mg/kg BID - SID)

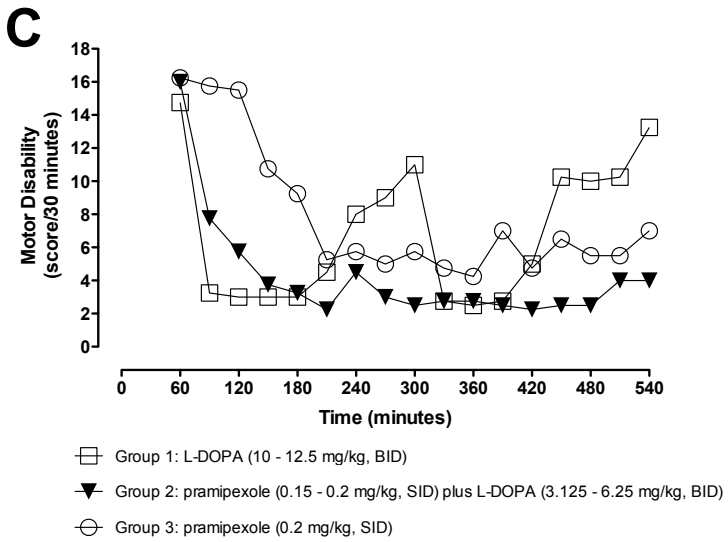

C
Motor Disability
(score/30 minutes)
18
16
14
12
10
8
6
4
2
0
0
60
120
180
240
300
360
420
480
540
Time (minutes)
Group 1: L-DOPA (10 - 12.5 mg/kg, BID)
Group 2: pramipexole (0.15 - 0.2 mg/kg, SID) plus L-DOPA (3.125 - 6.25 mg/kg, BID)
Group 3: pramipexole (0.2 mg/kg, SID)

Figure 6. 2 Total motor disability reversal (A) and mean timecourse (B&C) for PHASES I and II

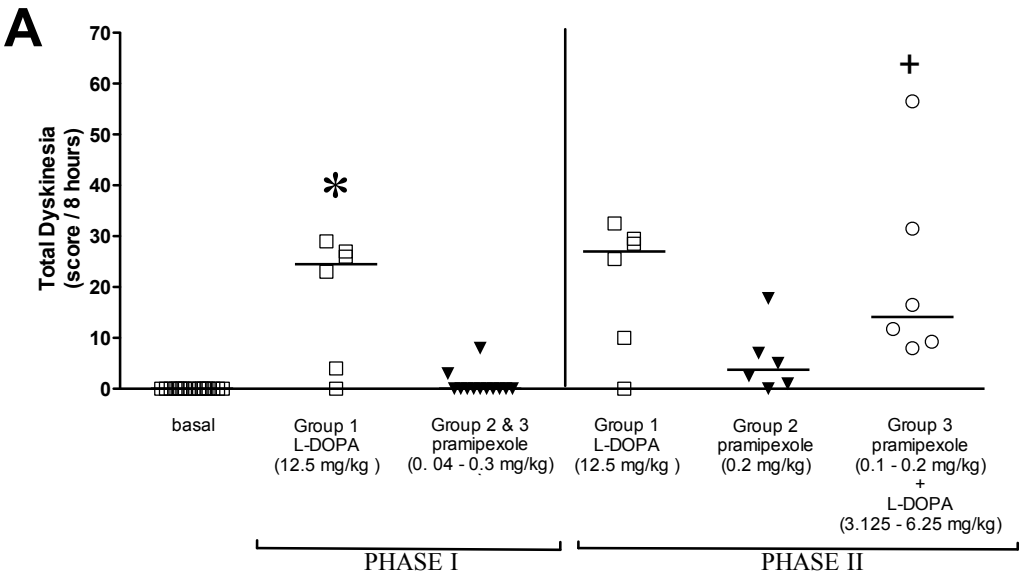
PHASE I: L-DOPA (12.5 mg/kg, p.o, plus carbidopa 12.5 mg/kg, p.o, BID, n=6); pramipexole (0.04 - 0.3 mg/kg, p.o, plus domperidone 2.0 mg/kg, p.o, BID – SID, n=12).

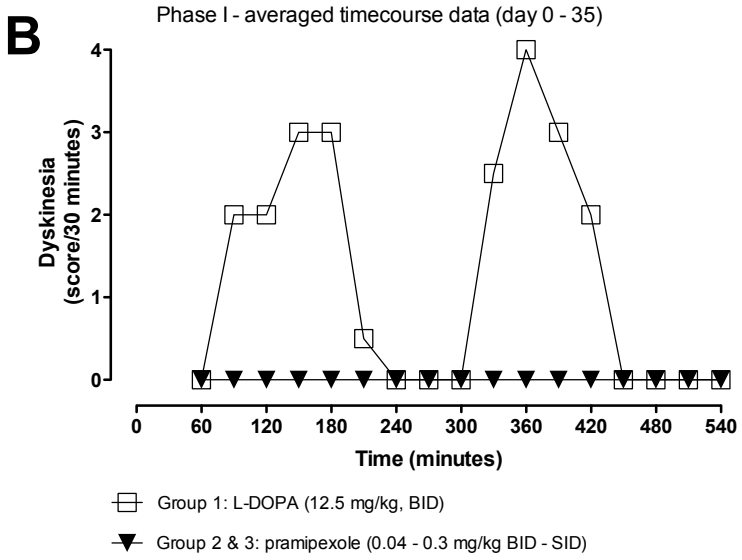
PHASE II: L-DOPA (10 - 12.5 mg/kg, p.o, plus carbidopa 12.5 mg/kg, p.o, BID, n=6); pramipexole (0.1 - 0.2 mg/kg, p.o, plus domperidone 2.0 mg/kg, p.o, SID, n=6) plus L-DOPA (3.125 – 6.25 mg/kg, p.o, plus carbidopa 12.5 mg/kg, p.o, BID); pramipexole (0.2 mg/kg, p.o, plus domperidone 2.0 mg/kg, p.o, SID, n=6).
Data (n = 6 - 12) are presented as the median and range of the motor disability scores of the averaged test days for PHASE I (days 7, 14, 21, 28, 35) and PHASE II (days 42, 49, 56 and 63) (A). Averaged timecourse data are presented as median values for Phase I (B) and Phase II (C). * indicates that motor disability was significantly improved compared to baseline motor disability scores for PHASE I. + indicates that motor disability following combined L-DOPA and pramipexole treatment was significantly improved compared to motor disability following L-DOPA alone for PHASE II ($p = 0.0260$) (Kruskal Wallis, $p < 0.0001$, Mann Whitney, $p < 0.05$).

6.4.3 Assessment of dyskinesia expression over PHASE I and II of the study

The administration of pramipexole alone (group 2/3) during PHASE I did not produce a significant increase in dyskinesia compared to baseline dyskinesia levels however, L-DOPA (group 1) administration did produce a significant increase in dyskinesia during PHASE I (figure 6.3A).

During PHASE II, L-DOPA (group 1) continued to produce similar dyskinesia levels as seen in PHASE I and pramipexole alone (group 2) did not produce significantly more severe dyskinesia levels compared to baseline. The administration of pramipexole plus L-DOPA (group 2) produced a significant increase in dyskinesia during PHASE II compared to pramipexole alone (group 2) (Figure 6.3C).


A
Total Dyskinesia (score / 8 hours)
70
60
50
40
30
20
10
0
*
+
basal
Group 1 L-DOPA (12.5 mg/kg)
Group 2 & 3 pramipexole (0. 04 - 0.3 mg/kg)
Group 1 L-DOPA (12.5 mg/kg)
Group 2 pramipexole (0.2 mg/kg)
Group 3 pramipexole (0.1 - 0.2 mg/kg) + L-DOPA (3.125 - 6.25 mg/kg)
PHASE I
PHASE II


B
Phase I - averaged timecourse data (day 0 - 35)
Dyskinesia (score/30 minutes)
4
3
2
1
0
0
60
120
180
240
300
360
420
480
540
Time (minutes)
Group 1: L-DOPA (12.5 mg/kg, BID)
Group 2 & 3: pramipexole (0.04 - 0.3 mg/kg BID - SID)

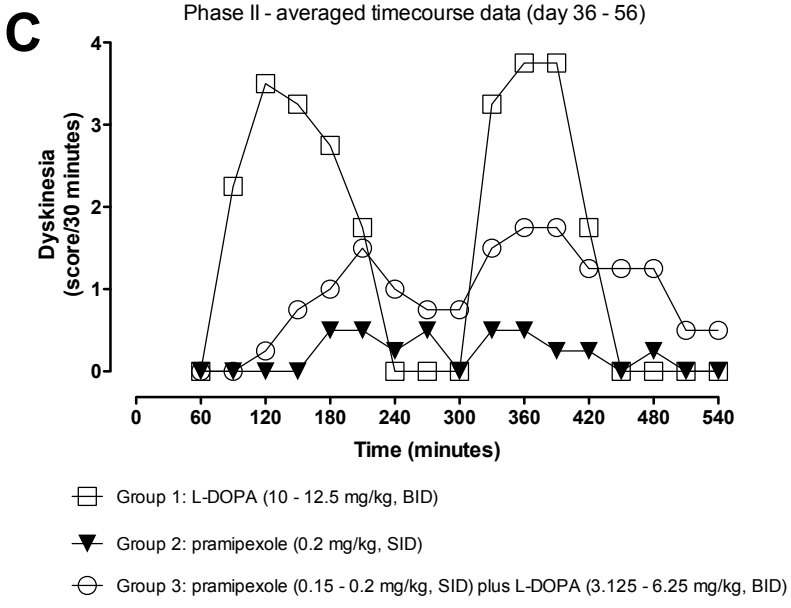

C
Phase II - averaged timecourse data (day 36 - 56)
Dyskinesia (score/30 minutes)
4
3
2
1
0
0
60
120
180
240
300
360
420
480
540
Time (minutes)
Group 1: L-DOPA (10 - 12.5 mg/kg, BID)
Group 2: pramipexole (0.2 mg/kg, SID)
Group 3: pramipexole (0.15 - 0.2 mg/kg, SID) plus L-DOPA (3.125 - 6.25 mg/kg, BID)

Figure 6. 3 The expression of dyskinesia totals (A) and mean timecourses (B&C) for PHASES I and II

PHASE I: L-DOPA (12.5 mg/kg, p.o, plus carbidopa 12.5 mg/kg, p.o, BID, n=6); pramipexole (0.04 - 0.3 mg/kg, p.o, plus domperidone 2.0 mg/kg, p.o, BID – SID, n=12).

PHASE II: L-DOPA (10 - 12.5 mg/kg, p.o, plus carbidopa 12.5 mg/kg, p.o, BID, n=6); pramipexole (0.1 - 0.2 mg/kg, p.o, plus domperidone 2.0 mg/kg, p.o, SID, n=6) plus L-DOPA (3.125 – 6.25 mg/kg, p.o, plus carbidopa 12.5 mg/kg, p.o, BID); pramipexole (0.2 mg/kg, p.o, plus domperidone 2.0 mg/kg, p.o, SID, n=6).
Data (n = 6 or 12) are presented as the median and range of the dyskinesia scores of the combined test days for PHASE I (days 7, 14, 21, 28, 35) and PHASE II (days 42, 49, 56 and 63). Averaged timecourse data are presented as median values for Phase I (B) and Phase II (C). *indicates that dyskinesia was significantly greater compared to baseline values for PHASE I. + indicates that dyskinesia following combined L-DOPA and pramipexole treatment was significantly greater than dyskinesia following pramipexole alone for PHASE II ($p = 0.0260$) (Kruskal Wallis, $p < 0.0001$, Mann Whitney, $p < 0.05$).

6.4.4 Assessment of peak dyskinesia over PHASE I and II of the study

During PHASE I peak dyskinesia was significantly increased following L-DOPA administration (group 1) compared to baseline dyskinesia levels for the animals prior to any drug administration. Pramipexole alone (group 2/3) did not induce significant dyskinesia expression compared to baseline levels (figure 6.4).

During PHASE II the peak L-DOPA induced dyskinesia seen in group 1 was similar to those seen in PHASE I (Figure 6.4). During PHASE II pramipexole alone (group 2) did not produce a significant increase in peak dyskinesia compared to pramipexole alone. Pramipexole plus L-DOPA (group 3) during PHASE II produced significantly higher peak dyskinesia levels compared to pramipexole alone (group 2), which was moderate in nature (score of 2) (Figure 6.4).

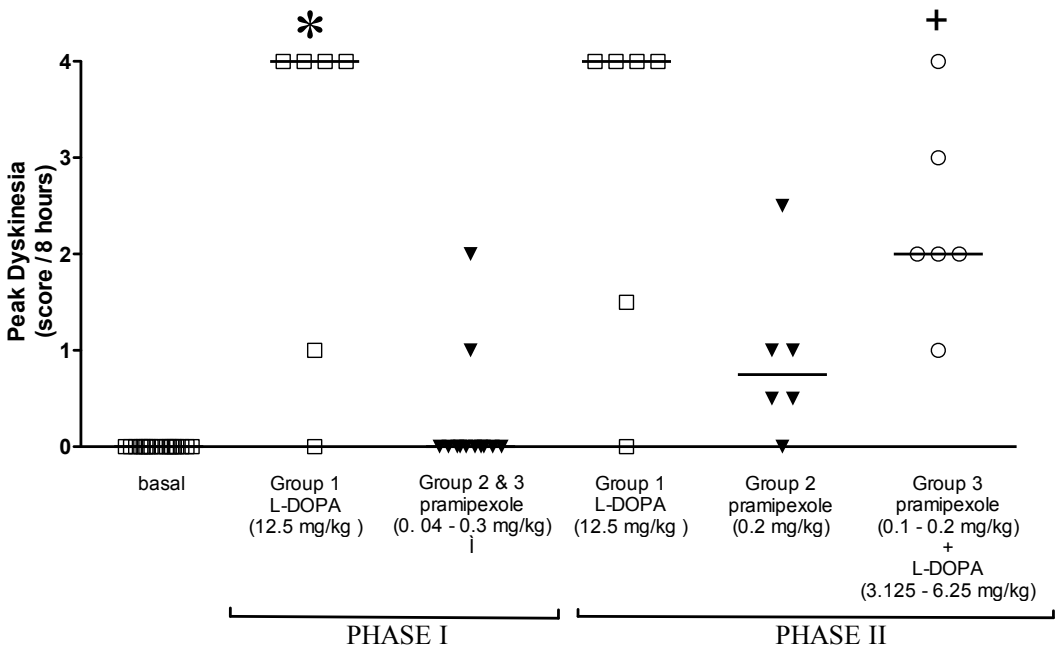


Figure 6. 4 The expression of peak dyskinesia totals for PHASES I and II of the study

PHASE I: L-DOPA (12.5 mg/kg, p.o, plus carbidopa 12.5 mg/kg, p.o, BID, n=6); pramipexole (0.04 - 0.3 mg/kg, p.o, plus domperidone 2.0 mg/kg, p.o, BID – SID, n=12).

PHASE II: L-DOPA (10 - 12.5 mg/kg, p.o, plus carbidopa 12.5 mg/kg, p.o, BID, n=6); pramipexole (0.1 - 0.2 mg/kg, p.o, plus domperidone 2.0 mg/kg, p.o, SID, n=6) plus L-DOPA (3.125 – 6.25 mg/kg, p.o, plus carbidopa 12.5 mg/kg, p.o, BID); pramipexole (0.2 mg/kg, p.o, plus domperidone 2.0 mg/kg, p.o, SID, n=6).
Data (n = 6 or 12) are presented as the median and range of the peak dyskinesia scores of the averaged test days for PHASE I (days 7, 14, 21, 28, 35) and PHASE II (days 42, 49, 56 and 63). * indicates that dyskinesia was significantly greater compared to basal values for PHASE I. + indicates that dyskinesia following combined L-DOPA and pramipexole treatment was significantly greater than dyskinesia following pramipexole alone for PHASE II (Kruskal Wallis, $p < 0.0001$, Mann Whitney, $p < 0.05$).

6.4.5 Behavioural assessment of acute L-DOPA challenges (Phase III) in previously treated groups (1-3) during Phase I and II

Following the end of the chronic dosing study with the three different treatment groups in PHASE II, animals were challenged with L-DOPA (12.5mg/kg, plus carbidopa 12.5mg/kg) once a week for three consecutive weeks. The administration of L-DOPA increased locomotor activity, reversed motor disability and induced dyskinesia in all three treatment groups, which were not significantly different between the three groups previously receiving different treatments (figure 6.5 A-G).

Although the peak dyskinesia produced by L-DOPA (12.5mg/kg, plus carbidopa 12.5mg/kg) was not significantly different between the three treatment groups previously receiving different treatments (Figure 6.1), the groups which had previously received either pramipexole alone (group 2) or pramipexole plus L-DOPA (group 3) tended to show lower dyskinesia levels despite not reaching statistical significance. Indeed group 2 animals previously receiving pramipexole alone, did not reach the most severe dyskinesia rating at any time after L-DOPA administration (figure 6.5G).

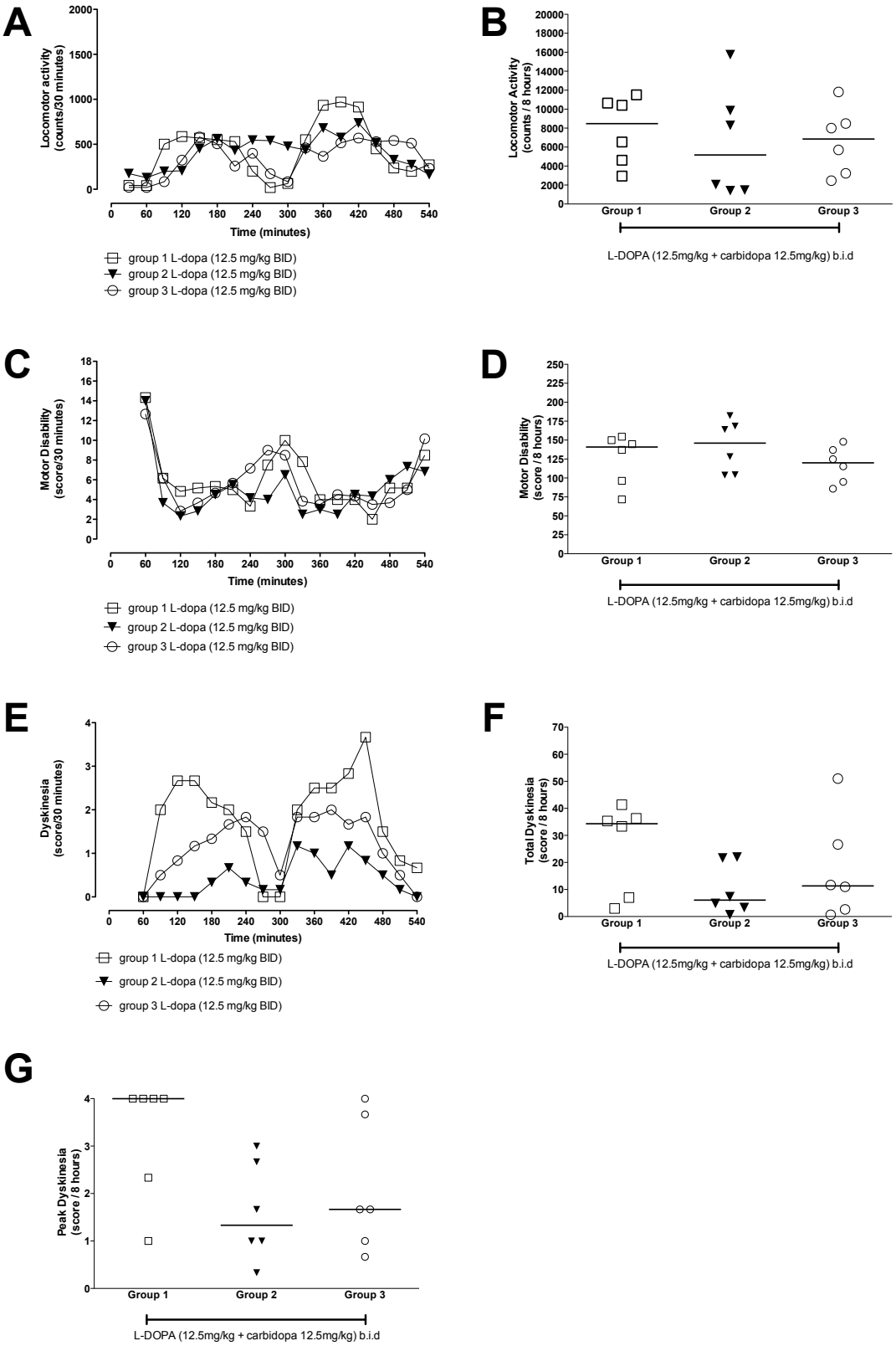

A
Locomotor activity (counts/30 minutes)
Time (minutes)
group 1 L-dopa (12.5 mg/kg BID)
group 2 L-dopa (12.5 mg/kg BID)
group 3 L-dopa (12.5 mg/kg BID)
B
Locomotor Activity (counts / 8 hours)
Group 1
Group 2
Group 3
L-DOPA (12.5mg/kg + carbidopa 12.5mg/kg) b.i.d
C
Motor Disability (score/30 minutes)
Time (minutes)
group 1 L-dopa (12.5 mg/kg BID)
group 2 L-dopa (12.5 mg/kg BID)
group 3 L-dopa (12.5 mg/kg BID)
D
Motor Disability (score / 8 hours)
Group 1
Group 2
Group 3
L-DOPA (12.5mg/kg + carbidopa 12.5mg/kg) b.i.d
E
Dyskinesia (score/30 minutes)
Time (minutes)
group 1 L-dopa (12.5 mg/kg BID)
group 2 L-dopa (12.5 mg/kg BID)
group 3 L-dopa (12.5 mg/kg BID)
F
Total Dyskinesia (score / 8 hours)
Group 1
Group 2
Group 3
L-DOPA (12.5mg/kg + carbidopa 12.5mg/kg) b.i.d
G
Peak Dyskinesia (score / 8 hours)
Group 1
Group 2
Group 3
L-DOPA (12.5mg/kg + carbidopa 12.5mg/kg) b.i.d

Figure 6. 5 The effect of L-DOPA on locomotor activity (A&B), motor disability (C&D), dyskinesia (E&F) and peak dyskinesia (G) averaged over 3 acute L-DOPA challenges when administered on 3 separate days of PHASE III following the cessation of the chronic treatments

L-DOPA (12.5 mg/kg + carbidopa 12.5mg/kg) b.i.d administered with a 4 hour interval. Data (n = 6 per group) are presented as the median and range of total locomotor activity, motor disability, dyskinesia and peak dyskinesia counts averaged over 3 acute L-DOPA challenges over 3 weeks starting 1 week after the end of PHASE II. Time course data (A, C & E) shows individual challenges. Totals data is presented as the average of the three challenges.
Group 1 previously received L-DOPA alone; Group 2 previously received pramipexole alone; Group 3 previously received pramipexole plus L-DOPA. There was no significant difference between all three treatment groups ($p > 0.05$).

6.5 Discussion

The previous studies in this thesis set out to investigate whether the efficacy of L-DOPA could be optimised by reducing the expression of dyskinesia, whilst maintaining optimal motor function. We have shown that subtle differences in the timing of administration, use and deployment of DDCIs and L-DOPA pro-drug formulations can improve L-DOPA efficacy in predictive animal models of Parkinson's disease. There is however, a need for better treatments, which prolong motor function and reduce dyskinesia expression. In this final chapter we designed a chronic dosing study to investigate whether combination of L-DOPA with the dopamine agonist, pramipexole, could reduce the priming of dyskinesia, the severity of dyskinesia and whether earlier use of dopamine agonists prior to L-DOPA combination offered therapeutic benefits using a switching study.

The first part of this study showed that both L-DOPA and pramipexole prime for dyskinesia confirming previous studies from these laboratories (Jenner 2000; Jackson et al. 2007; Stockwell et al. 2008). However, there was a significant difference in the severity of dyskinesia, measured as peak dyskinesia, during Phase I (figure 6.4A) whereby L-DOPA significantly induced greater peak dyskinesia compared to pramipexole alone. Despite producing equivalent improvements in motor function by dose titration in the early stages of the study (refer to table 6.1A), there was the rapid development of marked involuntary movements in response to L-DOPA that appeared with every dose administered but only mild dyskinesia in response to pramipexole treatment.

This difference may relate solely to difference in the duration of action (figure 6.1 B&C) of L-DOPA and pramipexole with the former resulting in pulsatile non-physiological stimulation of striatal dopamine receptors while the latter produces a more tonic physiological stimulation as suggested by the concept of continuous dopaminergic stimulation (Obeso et al. 1987; Stocchi et al. 2001; Honig et al. 2009b). However, since there is no correlation between the ability of dopamine agonists to prime / induce dyskinesia and their biological half-lives, another concept is that there are inherent differences between dopamine agonists that lead to low dyskinesia induction by some molecules.

Whether the difference between dopamine agonists and L-DOPA relates to the process of dyskinesia induction ('priming') or whether it has more to do with the ability to express dyskinesia is not well established. However, in previous studies, MPTP-treated marmosets have been treated with either ropinirole, piribedil or rotigotine resulting in mild dyskinesia and shown that switching to an equi-effective anti-parkinsonian dose of L-DOPA leads to the immediate expression of intense involuntary movements (Smith et al. 2006; Jackson et al. 2007; Stockwell et al. 2008). This suggests that agonists do prime for dyskinesia but do not express dyskinesia to the same degree as L-DOPA. For this reason the primary objective of this study was to determine whether combination treatment of L-DOPA and pramipexole could reduce dyskinesia expression and onset. The results of the second part of the study showed that a reduction in L-DOPA dosage concomitant with the introduction of pramipexole treatment

did not result in any reduction in motor function and in fact, there was a trend for an improvement (figure 6.2C). Importantly, however, dyskinesia diminished to a level that was ranked between that seen with treatment with L-DOPA alone and pramipexole alone (figure 6.3 A&C). This suggests that the concept of using a combination of L-DOPA and pramipexole in late stage disease may have therapeutic benefit. Precisely how the reduction in dyskinesia occurs is not certain. It could be the lowered ability of the agonist to express dyskinesia in L-DOPA primed animals or alternatively, since the study basically incorporated an L-DOPA 'sparing' strategy, it could simply reflect the lowering of the L-DOPA dosage. Overall pramipexole may not express dyskinesia to the same extent as L-DOPA but also it may not prime basal ganglia to the same extent as other dopamine agonists and may even have an effect on established priming when subsequently introduced in to L-DOPA exposed MPTP-treated primates.

The final investigation of this chapter was to assess whether early initiation with pramipexole results in lower dyskinesia levels once L-DOPA is combined to the treatment regimen. This was assessed by acute L-DOPA challenges at the end of chronic drug treatment (Phase II). The acute L-DOPA challenges (Phase III) initiated at one week after the cessation of the repeated treatment schedules showed no difference in the reversal of motor disability in the treatment groups but in both groups where the animals were treated with pramipexole alone (group 2) or a combination of L-DOPA and pramipexole (group 3), there was less dyskinesia. This appears to suggest that there is a subtle difference between initiating

treatments in late stage disease with a dopamine agonist versus L-DOPA. Whilst this did not reach statistical significance, it is in line with clinical studies, which show that dopaminergic treatment strategies, which reduce L-DOPA dose, can offer clinical benefit (Nissinen et al. 2009).

The present data add to and support previous preclinical and clinical observations in this area which have shown that treating otherwise drug naïve MPTP treated common marmosets with combinations of L-DOPA and the dopamine agonist ropinirole, could alter dyskinesia induction (Jackson et al. 2007). The use of an agonist dominant combination provided efficacy but levels of dyskinesia were not different from that produced by ropinirole alone whereas an L-DOPA dominant combination resulted in the expected marked dyskinesia seen with L-DOPA alone. We have also shown that in MPTP-treated common marmosets treated with L-DOPA to induce marked dyskinesia, switching to an equivalent anti-parkinsonian dose of ropinirole or piribedil led to an immediate reduction in dyskinesia intensity while the control of motor function was maintained (Smith et al. 2006).

All of these studies suggest that there is a role for dopamine agonists in the treatment of the later stages of Parkinson's disease as L-DOPA 'sparing' strategies.

The clinical value of combination therapy with L-DOPA and dopamine agonists in the later stages of the treatment of Parkinson's disease has also

been demonstrated. Subcutaneous or intravenous infusions of apomorphine and lisuride have been shown to reduce dyskinesia intensity in some patients with advanced Parkinson's disease who were also receiving L-DOPA therapy (Obeso et al. 1987; Bittkau et al. 1988; Baronti et al. 1992; Zaleska et al. 1999; Stocchi et al. 2001; Rudzinska et al. 2007). Similar results can be obtained using continuous intra-jejunal infusion of L-DOPA (Honig et al. 2009) suggesting that it is the continuous nature of drug delivery that can lead to the reversal of the expression of dyskinesia to dopaminergic medication.

What we have from the present study is the suggestion from a highly predictive model of the clinical actions of drugs in Parkinson's disease, that consideration should be given to reducing the optimal or stable doses of L-DOPA that are inevitably used in the later stages of the illness to determine whether replacement by pramipexole maintains the control of motor function but lessens dyskinesia intensity that might otherwise become treatment limiting. The other important element for clinical consideration is initiating dopamine agonist treatment earlier with the aim of reducing dyskinesia expression, peak dyskinesia and allowing a greater therapeutic window of opportunity for L-DOPA when combination therapy is required.

Chapter 7

General Discussion

7. General Discussion

7.1 Overview of thesis

Long after the initial discovery and use of L-DOPA to treat the symptomatic effects of Parkinson's disease, it still remains the treatment of choice despite its association with motor complications. However, there are a number of adjuncts used with L-DOPA, and alternative L-DOPA based therapies that may reduce the significance of these treatment-limiting complications. Therefore the hypothesis of this thesis was L-DOPA, the current 'gold standard' pharmacotherapy for Parkinson's disease can be improved by optimising its treatment strategies. In order to test this hypothesis a series of investigations were conducted to assess whether it is possible to:

- potentiate the clinical response of L-DOPA by maximising the efficiency of peripheral decarboxylase inhibition
- enhance the clinical response of L-DOPA through prodrug delivery
- optimise L-DOPA's clinical therapeutic window through combination therapy with dopamine agonists

7.2 Dopa-decarboxylase inhibitors and L-DOPA efficacy- summary of results

Overall in the 6-OHDA lesioned rat and MPTP-treated common marmoset model of Parkinson's disease, it was shown that that the efficacy of L-DOPA can be potentiated by improving the efficiency of peripheral and central DDCI. The use of the current DDCIs, carbidopa and benserazide was shown to be crucial in optimising the anti-Parkinson's disease effect of L-

DOPA by factoring in the timing of administration and dose of the DDCIs. Another noteworthy observation was that with the use of L-AMD, transitioning away from the traditional DDCI's used, could offer greater hope at maintaining motor function whilst reducing dyskinesia expression. Based on the results of this work, the hypothesis that we can optimise the pharmacotherapy of Parkinson's disease by optimising treatment strategies utilising DDCIs can be accepted, particularly dyskinesia reduction.

7.3 Pro-drug delivery strategies - summary of results

The novel L-DOPA prodrug, PRX 1354, showed improved L-DOPA induced behavioural responses in the MPTP-treated common marmoset in terms of motor disability although it did not have the same degree of improvement on dyskinesia expression.

Based on the results of this work, we can accept our hypothesis that we can enhance the clinical response to L-DOPA through prodrug delivery.

7.4 L-DOPA combination therapy with dopamine agonists - summary of results

In the MPTP-treated common marmoset model of Parkinson's disease, it was shown that L-DOPA combined with the dopamine agonist pramipexole resulted in improved motor function and a reduction in dyskinesia. Despite their being no difference in the ability of L-DOPA or pramipexole alone to prime for dyskinesia expression, there was a trend for dyskinesia

severity to be reduced in the combination treatment group when compared to L-DOPA alone. This highlights that optimising L-DOPA's clinical therapeutic window through combination therapy with dopamine agonists can offer benefits to Parkinson's disease patients providing that treatment has been personalised based on disease stage and current / previous treatment regimes.

Based on the results of this work, the hypothesis that we can optimise L-DOPA's clinical therapeutic window through combination therapy with dopamine agonists can be accepted.

7.5 Dopa-decarboxylase inhibitors and L-DOPA efficacy

From the investigations described in this thesis assessing the impact of DDCIs on dyskinesia expression in animal models of disease, several key questions have arisen:

1. Can the way DDCIs are used in the clinical setting be improved to offer greater therapeutic benefit of L-DOPA?
2. Do DDCIs have an impact on dyskinesia or disease progression?

7.5.1 Can the way DDCIs are used in the clinical setting be improved to offer greater therapeutic benefit of L-DOPA?

DDCI's were initially used to prevent L-DOPA associated peripheral side effects and reduce the total L-DOPA dose administered. Although this is still fundamental, this initial concept may have been too simplistic and

DDCIs could possibly help improve L-DOPA efficacy, ADME and avoidance of dyskinesia in a more robust and standalone fashion.

An important clinical parameter surrounding the use of DDCIs is there formulation with L-DOPA (Sinemet or Madopar). In the clinical setting the dose of L-DOPA is often titrated and adjusted to achieve a balanced and maximal response but it is unknown whether clinicians factor the change in the DDCI dose. Indeed the tablet formulation makes tailoring the dose difficult, and thus supplemental DDCI (e.g. Lodosyn - carbidopa) may be required.

In patients with advanced Parkinson's disease, the administration of L-DOPA without DDCI has been shown to improve motor fluctuations (Hironishi et al. 2002) possibly due to L-DOPA plasma levels having smaller fluctuations when L-DOPA is administered without DDCI rather than with a DDCI. It is not known why this is the case but it could be a combination of genetic variables such as up regulation of endogenous AADC or COMT enzymes. This does highlight that fixed dose L-DOPA combinations do not take into consideration the needs, disease stage and motor complications of the individual patients.

In the clinical setting, reports of worsening of the L-DOPA response over the day have been reported and this phenomenon needs further investigation (Bonuccelli et al. 2000). In other diseases such as Addison's disease, the timing of hydrocortisone administration is essential to be in rhythm with naturally rising levels of cortisol to have maximum efficacy but also to prevent side effects such as increased risk of osteoporosis and

type 2 diabetes (Jeffcoate. 1999). Whilst it is difficult to speculate whether there are detrimental effects to irregular timings of L-DOPA and or DDCI administration, more could be done to investigate this by using new technology such as 'chip in the pill' adherence recording methods (clinical trial number NCT01503008) such as Proteus. One possibility could be that early morning doses of L-DOPA plus DDCI has a very high and slow releasing DDCI component, which may help to compensate for the poor midday dose failure experienced by many patients in a similar fashion to seen with the supplemental dose of benserazide studies in the 6-OHDA lesioned rat (refer to chapter 3).

In 2013 Orion Pharmaceuticals received positive Phase II clinical date from a new L-DOPA/carbidopa/ entacapone combination ODM-101 (clinical trial number: NCT01766258). The key difference with this is the increased dose of carbidopa regardless of the L-DOPA dose. Whilst this is at odds with some of the findings in this thesis which show that increased carbidopa doses can induce greater dyskinesia expression, it is unknown what potential synergistic role the COMT inhibitor would have and whether inhibiting both the DDC and COMT pathways helps drive metabolism preferentially through one route over the other (e.g. inhibiting DDC by 100% leads to 50% greater than normal metabolism of L-DOPA via COMT). Other more simple explanations could be that increased peripheral inhibition of DDC would result in more L-DOPA being delivered centrally to be converted to DA.

7.5.2 Do DDCIs have an impact on dyskinesia or disease progression?

An area of DDCI efficacy, which has yet to be explored, is whether they could impact L-DOPA priming for dyskinesia. It has not as yet been investigated whether DDCIs could have an effect on dyskinesia priming. It has long been known that L-DOPA primes for dyskinesia expression to a greater degree than dopamine agonists and while the exact cause of this is not known there are beliefs that this is linked to the pulsatility of dopaminergic stimulation (Jenner et al. 2011) and receptor affinity. There have not been any studies to assess the effect of the DDCI in combination with L-DOPA in the priming process but *invivo* and studies manipulating the AADC gene have shown improvements in motor function (Lee et al. 2006). This does highlight that there is evidence for assessing the impact of DDCIs on dyskinesia expression. Our understanding and application of DDCIs and the AADC gene could potentially open new avenues of research and clinical drug candidates to help optimise the efficacy of L-DOPA by overcoming the failure to respond to L-DOPA because of a reduction in AADC activity which is profoundly decreased in the basal ganglia nigrostriatal nerve terminals of Parkinson's disease patients in the advanced stages of the disease. Other recent studies by Colamartino and colleagues, demonstrated that carbidopa is effective in reducing the damage caused by reactive oxygen intermediates both alone and in combination with L-DOPA (Colamartino et al. 2012). This again could imply that insufficient doses of DDCI could expose patients to greater cellular oxidative stress than is necessary and could slow the disease progress. Having greater flexibility in dosing regimens, which account for

DDCI therefore can only be a positive step forward and closer to the goal of personalized healthcare but this would not be without its own limitations. A poly-pharmacy or multi-drug approach, which increases pill burden but allows greater drug titrations could offer therapeutic benefit to patients who experience fluctuating L-DOPA responses. However, this may be impractical in the real world and increase the likelihood of non-adherence (Horne et al. 1999; Daley et al 2012). Thus combination therapy in one pill remains the most practical way forward.

7.5.3 Summary of DDCI effects in Parkinson's disease

Before the use and understanding of DDCIs can be utilised to optimise the clinical response of L-DOPA, the important role of DDCIs needs to be acknowledged, referring not to L-DOPA but L-DOPA plus DDCI.

Overall DDCIs remain a valid target for improving Parkinson's disease patient outcomes. Choice, dose, titration and chemical structures still need refining but support the hypothesis that we can optimise the current gold standard pharmacotherapy of Parkinson's disease.

7.6 Pro-drug delivery strategies

These studies suggesting that the use of a prodrug of L-DOPA can improve Parkinson's disease pharmacotherapy raise the following questions:

1. What do we need from new L-DOPA pro-drugs?
2. Do L-DOPA pro-drugs offer advantages over combination therapies?

7.6.1 What do we need from new L-DOPA prodrugs

The development of L-DOPA pro-drugs to achieve desired pharmacodynamic responses is still a tremendous challenge despite many laboratories investigating subtle chemical manipulations (Zhou et al. 2010; Bodor et al. 1977). The delivery of L-DOPA via a pro-drug approach is one that allows various factors such as PK, pulsatility of receptor stimulation, specificity for the transport receptors and also route and speed of metabolism to be explored.

The optimal PK profile of L-DOPA pro-drugs has been discussed previously (chapter 5), suggesting the need for increased duration of plasma exposure, reduced peripheral DDC and COMT metabolism and improved stability and solubility. However, approaches to prodrug design can be vastly variable in their attempt to achieve this. For example, designing a pro-drug, which is resistant to DDC in the periphery compared to a pro-drug which only work effectively if administered with the right dose of a DDCI. Whilst both options have positive and negative implications, the DDC resistant option maybe more valuable due to the scarcity of data available about DDCIs on dyskinesia priming, genetic makeup of patients who have irregular DDC levels and also the 'cost' of making a multi-drug formulation being more expensive.

Other options, which avoid metabolism issues, could be designing receptor-activated compounds e.g. L-DOPA prodrugs, which firstly stimulate the dopamine receptors but avoid dopamine release from 5-HT terminals (Carta et al. 2007) and are subsequently metabolised to release L-DOPA may offer promise.

Other more successful options both in the laboratory and at a clinical level have been to develop new and improved routes of administration (e.g. dopamine agonist patches). Using optimal routes of administration with L-DOPA prodrugs may yield even further advancements in our symptomatic management of Parkinson's disease.

7.6.2 Do L-DOPA pro-drugs offer advantages over combination therapies

Advances in our understanding of the complications of dopamine replacement therapy and delivery have led to an appreciation of the opportunities for novel modes of drug delivery and formulation in Parkinson's disease.

One interesting approach could be the combination of DDCI and L-DOPA into a single chemical entity whereby the DDCI is rapidly metabolised and followed by slow release and fast acting L-DOPA. One compound, which has factored some of these elements, is IPX066, which is different to other drug formulations in that it contains special beads designed to dissolve at different rates within the stomach and the intestines (Mao et al. 2013). This was designed to provide longer lasting benefit for patients with Parkinson's disease (Yacoubian. 2013). Whilst the results for IPX066 have shown promise in the clinical trial setting, the reported adverse events have meant that the FDA have ruled its cardiovascular disease risk to high. Other novel pro-drug drugs part of the IPX series, include IPX750. This drug is a dopaminergic prodrug designed to retain stereospecific binding at the glucose and dopamine transporter and at dopaminergic

receptors. In preclinical studies, IPX750 showed anti-Parkinson activity in three different Parkinson's disease rodent models (Jiang et al. 2004). This compound raises interesting possibilities for L-DOPA pro-drugs whereby our understanding of where L-DOPA is absorbed from the GI tract, could allow us to develop compounds which have greater affinity and faster circulation through the systemic circulation to the get to the specific target area of the GI tract.

7.6.3 Summary of pro-drug strategies

Although prodrugs like PRX 1354 alone may not offer great hope for patients, the combined information on chemical structures and efficacy could prove to be valuable in the future. Overall L-DOPA prodrugs remain a valid target for improving Parkinson's disease patient outcomes but must be assessed continuously with other changes in the treatment landscape including route of administration to support the hypothesis that we can optimise the current gold standard pharmacotherapy of Parkinson's disease.

7.7 L-DOPA combination therapy with dopamine agonists

From the investigations in these studies, several questions arise. These questions are:

1. Does the timing of dopamine agonist treatment initiation have an effect on dyskinesia?

2. Is dyskinesia better tackled via multiple drugs with multiple mechanisms of action?

The following sections discuss the future ideas around the use of combination therapy with L-DOPA that have arisen from this work.

7.7.1 Does the timing of dopamine agonist treatment initiation have an effect on dyskinesia

Dopamine receptor agonists were originally employed as adjunctive or "add on" medications to supplement the use of L-DOPA when further dopaminergic effect was required or when treatment complications arose. Recent studies have confirmed the effectiveness of using agonists as monotherapy in early Parkinson's disease which is particularly important for young-onset Parkinson's disease patients, as this group is at greater risk for the development of motor fluctuations and dyskinesia after relatively short term use of L-DOPA.

Whilst the primary focus of combination therapy is to improve the clinical efficacy of the treatment regimen, it is often overlooked in terms of increasing the duration of the therapeutic window of drug activity overall. However, it may be that the use of dopamine agonists throughout the disease course may improve Parkinson's disease pharmacotherapy throughout the course of the disease.

Other approaches to increase the therapeutic window could be via pro-drug approaches, which combine L-DOPA with dopamine agonists in the

same chemical structure to provide the long and short acting dopaminergic stimulation. This could even go beyond two drug combinations. It could be inclusive of MAO-B inhibitors and COMT inhibitors. It is often thought that single drugs formulations treat a disease but to treat a syndrome such as Parkinson's disease you may have to deploy a large variety of different acting drugs to have an overall effect.

7.7.2 Is dyskinesia better tackled via multiple drugs with multiple mechanisms of action

An interesting point is whether the dyskinesia induced by dopamine agonists is the same as L-DOPA? Whilst the presentation clinically may be similar or identical, is the mechanism of dyskinesia expression or priming the same? Given some of the differences in the clinical manifestations of dyskinesias, it is likely that distinct mechanisms underlie the different types of dyskinesia. For example, in peak-dose dyskinesia, the key abnormality is felt to be over activation of the direct pathway that leads to under activity of basal ganglia output (Hashimoto et al. 2012). However, distinct activation patterns involving GPe and STN in the direct pathway lead to chorea or dystonia (Sanghera et al. 2003). Given the multiple variances of dyskinesia, different therapeutic approaches may be required based on the predominant type of phenomenology. Increasing our understanding of dyskinesia presentation, type and activation may lead us to developing strategies, which help to identify dyskinesia more efficiently and provide the most appropriate treatment. In this respect if dyskinesia

induced by dopamine agonists were to be different to L-DOPA then we could develop regimens, which factor this element.

It has long been established that L-DOPA efficacy for advanced Parkinson's disease treatment is superior to dopamine agonists both in terms of improvement of motor deficits and non-motor side effects (Maratos et al. 2001; Bittkau et al. 1988; Honig et al. 2009). However, the therapeutic window of maximal benefit with L-DOPA is limited and strategies to manage motor complications include delaying the initiation of levodopa therapy when possible, altering levodopa doses or frequency of administration, and adding adjunct therapies such as COMT inhibitors, dopamine agonists, MAO-B inhibitors and amantadine as other candidates for adjunct treatments.

So when considering combination therapies with L-DOPA, both the short term and long term efficacy needs to be addressed. There is no defined treatment pathway, which accounts for disease stage and rate of progression at an individual patient level and the treatment strategy sits with the experience and understanding of clinicians. Historical experience often overrides tailored patient- based therapeutics taking into consideration individual patient needs. Improving and developing diagnostic tools, which help achieve 'personalised medicine' for Parkinson's disease patients will, in the future, improve Parkinson's disease therapy in similar ways to other disease areas such as identifying HER2+ breast cancer patients allows tailored treatment.

7.8 Limitations and critique

Whilst the investigations described in this thesis have helped to address some fundamental questions about L-DOPA therapy in Parkinson's disease using animal models of the disease, these studies were not without their limitations.

Firstly, both the 6-OHDA lesioned rat and MPTP-treated common marmoset failed to display some of the fundamental characteristics of Parkinson's disease in man, such as progressive degeneration of the dopaminergic system as well as other neurotransmitter systems such as 5-HT, noradrenaline and acetylcholine (Morin et al. 2013). They also do not express some key cardinal signs of the disease such as resting tremor, Lewy body neuropathology and neuropsychiatric alterations (which may be present but as yet there is no globally accepted scale to validate them). Animal models displaying the progressive neuronal degeneration have the potential to identify disease modifying / neuroprotective / neurorescue strategies. However, it is likely that the progressive nature of the disease contributes to the onset motor complications as these are not seen with high dose L-DOPA therapy in primates and are rarely seen in dopaminergic treatment of restless leg syndrome (Becker et al. 1993) or L-DOPA responsive dystonia. Models that show progressive neuronal degeneration including the Aphakia mice (Van den Munckhof et al. 2003) and MitoPark models (Ekstrand et al. 2009) may be important in understanding the role of disease progression on dyskinesia priming. Both of these models display a progressive loss of dopaminergic neurones and the MitoPark

model also displays intraneuronal inclusion bodies, although not containing α–synuclein, are more reflective of the DA loss and neuropathology in Parkinson's disease patients. However, they may help to identify whether using DDCIs or dopamine agonists to optimise L-DOPA treatment very early on in disease may improve motor function without inducing dyskinesia.

The predictive value of the MPTP-treated common marmoset has been valuable over the past 30 years in helping to increase our knowledge of the disease and further our understanding of treatment options and associated side effects. The 6-OHDA lesioned rat model is a useful screening tool to help identify compounds, which have an effect on the dopaminergic neurones. However, no models have been shown to be 100% predictive of effects in man (Blandini and Armentero. 2012). There have also been additional scales of observation developed to help gain more value from the 6-OHDA model by assessing motor function and dyskinesia expression with the use of AIMs (abnormal involuntary movements scale) (Lunblad et al. 2005), although these were not used in these studies. Whilst the AIMs model is not in its infancy any longer, it does require constant re-validation in every lab with new changes in environment, equipment and experimenters, which leaves greater room for variability between groups. More established primate models such as the MPTP-common marmoset model are more predictable and reliable even between different research groups.

Reductions in dyskinesia severity have been reported in animal models with dopaminergic agents such as ropinirole (Zubair et al. 2007) and some

non-dopaminergic agents like levetiracetam (Bezard et al. 2004) and yet the vast majority of the compounds / drugs in the clinical setting have failed to show improvements in dyskinesia, or only very minor improvements (Zesiewicz et al., 2005). This raises the question as to whether the animal models are predictive of clinical efficacy relevant. The reality is that we can't know with any certainty from the work in these animal models whether an effect will translate into positive clinical outcomes. However, we can ensure that we factor in as many variables as possible to produce robust data. An area, which is often forgotten about in chronic dosing studies, is 'does this adherence to medication in a lab correspond to the real world clinical setting'?

We know from the World Health Organisation that approximately 50% of patients are non-adherent with the medication as prescribed for long-term chronic conditions (Elm et al. 2007). Therefore it does not make sense to test compounds chronically and adherently in the lab or under clinical trial conditions because these results would not reflect actual dosing in the real world. Even the timing of drug administration, which we know from the lunchtime dose still challenges our management of Parkinson's disease but yet is not factored in under clinical test conditions. Should this thinking also be applied to clinical trial design in Parkinson's disease it may help to answer why many patients do not respond to pharmacotherapy in the real world compared to controlled conditions (Davis et al. 2010).

One area, which ensures consistency between pre-clinical and clinical testing is the standardisation of routes of administration and ensuring the

correct dose, is administered. Over the past 10 years we have seen the introduction of various new routes of administration including subcutaneous delivery of dopamine agonists like apomorphine (Antonini et al. 2011), transdermal application of synthetic dopamine agonists such as rotigotine (Neupro) (Waters. 2013) and intra-duodenal administration of Duodopa (Zibetti et al. 2013). Indeed, recently apomorphine has also been developed as an inhaled treatment regimen (Grosset et al. 2013), which avoids various metabolism related side effects including possible hepatic first pass metabolism but more importantly does not require injection subcutaneously and this has a positive impact on patients experience.

Whilst the focus of the work in this thesis has been on optimising L-DOPA treatment strategies, an area of work, which could provide further value, would be the investigation into improved outcomes following alternative routes of administration. For example intranasal administration of DDCIs could offer greater value than current oral administration? In fact in recent years, studies have shown that intranasal administration of L-DOPA (Lee et al. 2013) provides superior L-DOPA PK and AUC profiles compared to oral administration particularly in the absence of DDCI.

The work contained in this thesis has been based on observational responses in predictive animal models of Parkinson's disease to measure specific outcomes of L-DOPA induced behaviour i.e. motor disability and dyskinesia. The findings that the efficacy of L-DOPA can be improved with altered treatment strategies would be more robust if pharmacokinetic

analysis had been performed. However, whilst there were no pharmacokinetic experiments conducted in these studies, the focus of this work has been on behavioural responses, which optimise motor control in animal models and thereafter may allow some of the biochemical explanations to be inferred from these behavioural responses.

7.9 Future of Parkinson's disease treatment

The emerging understanding of the complex pathophysiology underlying dyskinesia will guide future efforts directed at prevention and treatment of drug-induced dyskinesia.

The pharmacotherapeutic management will also be further advanced in line with new genetic discoveries which have helped to identify specific gene loci which play a more critical role in drug efficacy (Droździk et al. 2013). Developing improved diagnostic tools, which utilise this type of information in the future may help to determine optimal treatment pathways for patients with improved outcomes but the investigations into neuroprotective or even neurorestorative therapies should not be ignored.

Improved digital (chip in the pill), phenotypic (different dyskinesia types) and surgical techniques (fetal / stem cell transplants) (Nishimura and Takahashi. 2013) will increase our understanding and capabilities at treating this syndrome and potentially help us to manage the condition more effectively and restore 'normal' motor function without treatment limiting dyskinesia and potentially lead to neuroprotection treatment

strategies.

The value of stem cells and gene therapy in future years may be more fruitful in developing new treatments and even cures for Parkinson's disease but until then, we must utilise the drugs and treatment regimens to the best of their ability and apply the knowledge we currently have to ensure we optimise patient outcomes, improve the quality of life and prepare ourselves for a growing number of patients inflicted by the disease James Parkinson described as

"the hand failing to answer with exactness the dictates of the will".

7.10 Final Conclusion

In the studies described in this thesis as well as other on-going research suggest that, even after 40 years in clinical use, L-DOPA remains the gold standard treatment for Parkinson's disease. However, optimising our treatment strategies could raise 'the bar' of the gold standard pharmacotherapy in Parkinson's disease and increase quality of life for patients.

The results presented in this thesis readdressed some fundamental questions in the continued search for improved outcomes for Parkinson's disease patients. Improved use of decarboxylase inhibitors, better design of prodrugs and DA agonist combination therapy may improve the control of motor disability and reduce the severity of dyskinesia, resulting in improved clinical outcomes. Thus, the hypothesis that L-DOPA, the current

'gold standard' pharmacotherapy for Parkinson's disease can be improved by optimising its treatment strategies can be accepted, however, there are still fundamental questions that need to be answered to address the disease process itself.

8. References

Admani AK, Verma S, Cordingley GJ, and Harris RI (1985) Patient benefits of l-dopa and a decarboxylase inhibitor in the treatment of Parkinson's disease in elderly patients. Pharmatherapeutica 4: 132-140

Ahlskog JE (2003) Parkinson's disease: is the initial treatment established? Curr Neurol Neurosci Rep 3: 289-295

Albani C, Zangger I, and Fischer G (1992) [Subcutaneous apomorphine infusion in the treatment of Parkinson disease]. Schweiz Rundsch Med Prax 81: 1011-1015

Albin RL, Young AB, Penney JB (1989) The functional anatomy of basal ganglia disorders. Trends Neurosci. 1989 Oct;12(10):366-75. Review

Anderson PO, Chen S, Paulsson KM, Sjögren HO, Li S (2001) Inhibition of the transcription factors AP-1 and NF-kappaB in CD4 T cells by peroxisome proliferator-activated receptor gamma ligands. Int Immunopharmacol. 2001 Apr;1(4):803-12

Antonini A, Isaias IU, Rodolfi G, Landi A, Natuzzi F, Siri C, Pezzoli G (2011) A 5-year prospective assessment of advanced Parkinson disease patients treated with subcutaneous apomorphine infusion or deep brain stimulation. J Neurol. 2011 Apr;258(4):579-85

Arai A, Kannari K, Shen H, Maeda T, Suda T, and Matsunaga M (2003) Amantadine increases L-DOPA-derived extracellular dopamine in the striatum of 6-hydroxydopamine-lesioned rats. Brain Res 972: 229-234

Arai E, Arai M, Uchiyama T, Higuchi Y, Aoyagi K, Yamanaka Y, Yamamoto T, Nagano O, Shiina A, Maruoka D, Matsumura T, Nakagawa T, Katsuno T, Imazeki F, Saeki N, Kuwabara S, Yokosuka O (2012) Subthalamic deep brain stimulation can improve gastric emptying in Parkinson's disease. Brain. 2012 May;135(Pt 5):1478-85

Araki T, Tanji H, Kato H, and Itoyama Y (1998) Sequential changes of dopaminergic receptors in the rat brain after 6-hydroxydopamine lesions of the medial forebrain bundle. J Neurol Sci 160: 121-127

Avorn J, Schneeweiss S, Sudarsky LR, Benner J, Kiyota Y, Levin R, and Glynn RJ (2005) Sudden uncontrollable somnolence and medication use in Parkinson disease. Arch Neurol 62: 1242-1248

Bakke OM and Scheline RR (1974) Inhibition of a minor pathway of L-dopa metabolism in the intestinal lumen using a decarboxylase inhibitor (Ro 4-4602). J Pharm Pharmacol 26: 377-379

Bankiewicz KS, Pivirotto P, Sanchez-Pernaute R, and Major EO (2001) Grafting genetically engineered cells into the striatum of nonhuman primates. Methods Mol Med 62: 269-278

Barone P (2003) Clinical strategies to prevent and delay motor complications. Neurology 61: S12-S16

Baronti F, Mouradian MM, Davis TL, Giuffra M, Brughitta G, Conant KE, and Chase TN (1992) Continuous lisuride effects on central dopaminergic mechanisms in Parkinson's disease. Ann Neurol 32: 776-781

Bartholini G and PLETSCHER A (1975) Decarboxylase inhibitors. Pharmacol Ther B 1: 407-421

Bartholini G, Pletscher A (1968) Cerebral accumulation and metabolism of C14-dopa after selective inhibition of peripheral decarboxylase. J Pharmacol Exp Ther. 1968 May;161(1):14-20

Bartoszyk GD, van AC, Bottcher H, and Seyfried CA (2003) EMD 281014, a new selective serotonin 5-HT2A receptor antagonist. Eur J Pharmacol 473: 229-230

Becker PM1, Jamieson AO, Brown WD (1993) Dopaminergic agents in restless legs syndrome and periodic limb movements of sleep: response and complications of extended treatment in 49 cases. Sleep. Dec;16(8):713-6.

Ben LH, Zhao J, Xin SM, Luo SQ, and Pei G (1999) Attenuation of delta opioid receptor-mediated signaling by kainic acid in neural cells: involvement of protein kinase C and intracellular Ca2+. Neuropharmacology 38: 991-998

Bergman H, Feingold A, Nini A, Raz A, Slovin H, Abeles M, and Vaadia E (1998) Physiological aspects of information processing in the basal ganglia of normal and parkinsonian primates. Trends Neurosci 21: 32-38

Betarbet R, Sherer TB, and Greenamyre JT (2002) Animal models of Parkinson's disease. Bioessays 24: 308-318

Bezard E, Brotchie JM, and Gross CE (2001) Pathophysiology of levodopa-induced dyskinesia: potential for new therapies. Nat Rev Neurosci 2: 577-588

Bittkau S and Przuntek H (1988) Chronic s.c. lisuride in Parkinson's disease--motor-performance and avoidance of psychiatric side effects. J Neural Transm Suppl 27: 35-54

Bloem BR, Irwin I, Buruma OJ, Haan J, Roos RA, Tetrud JW, and Langston JW (1990) The MPTP model: versatile contributions to the treatment of idiopathic Parkinson's disease. J Neurol Sci 97: 273-293

Bodor N, Sloan KB, Higuchi T, and Sasahara K (1977) Improved delivery through biological membranes. 4. Prodrugs of L-dopa. J Med Chem 20: 1435-1445

Bondiolotti GP, Galva MD, Villa F, Sciaba L, and Picotti GB (1995) In vitro effects on monoamine uptake and release by the reversible monoamine oxidase-B inhibitors lazabemide and N-(2-aminoethyl)-p-chlorobenzamide: a comparison with L-deprenyl. Biochem Pharmacol 50: 97-102

Bonifati V, Rizzu P, Squitieri F, Krieger E, Vanacore N, van Swieten JC, Brice A, van Duijn CM, Oostra B, Meco G, and Heutink P (2003) DJ-1(PARK7), a

novel gene for autosomal recessive, early onset parkinsonism. Neurol Sci 24: 159-160

Bonina F, Puglia C, Rimoli MG, Melisi D, Boatto G, Nieddu M, Calignano A, La Rana G and De Caprariis P (2003) Glycosyl derivatives of dopamine and L-dopa as anti-Parkinson prodrugs: synthesis, pharmacological activity and in vitro stability studies. J Drug Target. Jan;11(1):25-36

Bonnet AM, Houeto JL (1999) Pathophysiology of Parkinson's disease. Biomed Pharmacother. 1999 Apr;53(3):117-21.

Bonuccelli U, Del Dotto P, Lucetti C, Petrozzi L, Bernardini S, Gambaccini G, Rossi G and Piccini P (2000) Diurnal motor variations to repeated doses of levodopa in Parkinson's disease. Clin Neuropharmacol. 2000 Jan-Feb;23(1):28-33

Bonuccelli U, Colzi A, and Del DP (2002) Pergolide in the treatment of patients with early and advanced Parkinson's disease. Clin Neuropharmacol 25: 1-10

Boomsma F, van der Hoorn FA, and Schalekamp MA (1986) Determination of aromatic-L-amino acid decarboxylase in human plasma. Clin Chim Acta 159: 173-183

Boudin G, Pepin B, Guillard A, Godlewski S, Fabiani JM, and Haguenau M (1974) [Results after 3 to 5 years of treatment of 219 cases of Parkinson's

syndrome treated by L-Dopa and an L-Dopa -decarboxylase inhibitor combination]. Rev Neurol (Paris) 130: 273-284

Boulamery A, Simon N, Vidal J, and Bruguerolle B (2010) Effects of L-Dopa on circadian rhythms of 6-OHDA striatal lesioned rats: a radiotelemetric study. Chronobiol Int 27: 251-264

Breese GR and Traylor TD (1971) Depletion of brain noradrenaline and dopamine by 6-hydroxydopamine. Br J Pharmacol 42: 88-99

Brocks DR (1999) Anticholinergic drugs used in Parkinson's disease: An overlooked class of drugs from a pharmacokinetic perspective. J Pharm Pharm Sci 2: 39-46

Brooks DJ, Leinonen M, Kuoppamäki M, Nissinen H (2008) Five-year efficacy and safety of levodopa/DDCI and entacapone in patients with Parkinson's disease. J Neural Transm. 2008 Jun;115(6):843-9

Brod LS, Aldred JL, and Nutt JG (2012) Are high doses of carbidopa a concern? A randomized, clinical trial in Parkinson's disease. Mov Disord 27: 750-753

Brotchie JM and Fox SH (1999) Quantitative assessment of dyskinesias in subhuman primates. Mov Disord 14 Suppl 1: 40-47

Buck K and Ferger B (2008) Intrastriatal inhibition of aromatic amino acid decarboxylase prevents l-DOPA-induced dyskinesia: a bilateral reverse in

vivo microdialysis study in 6-hydroxydopamine lesioned rats. Neurobiol Dis 29: 210-220

Burkard WP, Gey KF, and Pletscher A (1962) A new inhibitor of decarboxylase of aromatic amino acids. Experientia 18: 411-412

Burkard WP, Gey KF, and Pletscher A (1963) Diamine oxidase in the brain of vertebrates. J Neurochem 10: 183-186

Burns RS, Chiueh CC, Markey SP, Ebert MH, Jacobowitz DM, and Kopin IJ (1983) A primate model of parkinsonism: selective destruction of dopaminergic neurons in the pars compacta of the substantia nigra by N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Proc Natl Acad Sci U S A 80: 4546-4550

Calne DB and Mizuno Y (2004) The neuromythology of Parkinson's Disease. Parkinsonism Relat Disord 10: 319-322

Campos-Romo A (2008) [Evaluation of motor disorders in animal models of Parkinson's disease]. Rev Neurol 46: 167-174

Carey RJ (1991) Chronic L-dopa treatment in the unilateral 6-OHDA rat: evidence for behavioral sensitization and biochemical tolerance. Brain Res 568: 205-214

Carey RJ (1991) Naloxone reverses L-dopa induced overstimulation effects in a Parkinson's disease animal model analogue. Life Sci. 1991;48(13):1303-8.

Carey RJ (1993) Motoric sensitization and levodopa accumulation after chronic levodopa treatment in an animal model of Parkinson's disease. J Geriatr Psychiatry Neurol 6: 152-160

Carey RJ, Pinheiro-Carrera M, Tomaz C, and Huston JP (1994) Early L-dopa treatment initially retards but later enhances dopamine receptor supersensitivity following unilateral dopamine denervation. Brain Res 658: 145-154

Carta M1, Carlsson T, Kirik D, Björklund A (2007) Dopamine released from 5-HT terminals is the cause of L-DOPA-induced dyskinesia in parkinsonian rats. Brain. Jul;130(Pt 7):1819-33.

Castagnoli N, Jr., Petzer JP, Steyn S, Castagnoli K, Chen JF, Schwarzschild MA, and Van der Schyf CJ (2003) Monoamine oxidase B inhibition and neuroprotection: studies on selective adenosine A2A receptor antagonists. Neurology 61: S62-S68

Cathala L, Guyon A, Eugene D, and Paupardin-Tritsch D (2002) Alpha2-adrenoceptor activation increases a cationic conductance and spontaneous GABAergic synaptic activity in dopaminergic neurones of the rat substantia nigra. Neuroscience 115: 1059-1065

Cavalli A, Bolognesi ML, Minarini A, Rosini M, Tumiatti V, Recanatini M, and Melchiorre C (2008) Multi-target-directed ligands to combat neurodegenerative diseases. J Med Chem 51: 347-372

Cenci MA, Lee CS, and Bjorklund A (1998) L-DOPA-induced dyskinesia in the rat is associated with striatal overexpression of prodynorphin- and glutamic acid decarboxylase mRNA. Eur J Neurosci 10: 2694-2706

Cenci MA and Lundblad M (2006) Post- versus presynaptic plasticity in L-DOPA-induced dyskinesia. J Neurochem 99: 381-392

Cenci MA, Whishaw IQ, and Schallert T (2002) Animal models of neurological deficits: how relevant is the rat? Nat Rev Neurosci 3: 574-579

Chaudhuri KR, Odin P, Antonini A, Martinez-Martin P (2011) Parkinson's disease: the non-motor issues. Parkinsonism Relat Disord. 2011 Dec;17(10):717-2

Chase TN, Baronti F, Fabbrini G, Heuser IJ, Juncos JL, and Mouradian MM (1989) Rationale for continuous dopaminomimetic therapy of Parkinson's disease. Neurology 39: 7-10

Christiaans, J.A.M. and Timmerman, H. (1996) Cardiovascular hybrid drugs: combination of more than one pharmacological property in one single molecule. European Journal of Pharmaceutical Sciences, 4, 1-22

Chritin M, Blanchard V, Raisman-Vozari R, Feuerstein C, Agid Y, Javoy-Agid F, and Savasta M (1996) DA uptake sites, D1 and D2 receptors, D2 and preproenkephalin mRNAs and Fos immunoreactivity in rat striatal subregions after partial dopaminergic degeneration. Eur J Neurosci 8: 2511-2520

Close SP, Marriott AS, and Pay S (1985) Failure of SKF 38393-A to relieve parkinsonian symptoms induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in the marmoset. Br J Pharmacol 85: 320-322

Cotzias GC, Papavasiliou PS, Gellene R (1969) Modification of Parkinsonism--chronic treatment with L-dopa. N Engl J Med.

Crevoisier C, Zerr P, Calvi-Gries F, and Nilsen T (2003) Effects of food on the pharmacokinetics of levodopa in a dual-release formulation. Eur J Pharm Biopharm 55: 71-76

Crossman AR (2000) Functional anatomy of movement disorders. J Anat. 2000 May;196 (Pt 4):519-25.

Da PM, Kettler R, Keller HH, Bonetti EP, and Imhof R (1987a) Ro 16-6491: a new reversible and highly selective MAO-B inhibitor protects mice from the dopaminergic neurotoxicity of MPTP. Adv Neurol 45: 175-178

Da PM, Kettler R, Zurcher G, Schaffner R, and Haefely WE (1987b) Inhibition of decarboxylase and levels of dopa and 3-O-methyldopa: a comparative study of benserazide versus carbidopa in rodents and of Madopar standard versus Madopar HBS in volunteers. Eur Neurol 27 Suppl 1: 9-20

Daley DJ, Myint PK, Gray RJ, Deane KH (2012) Systematic review on factors associated with medication non-adherence in Parkinson's disease. Parkinsonism Relat Disord. 2012 Dec;18(10):1053-61

Davis GC, Williams AC, Markey SP, Ebert MH, Caine ED, Reichert CM, and Kopin IJ (1979) Chronic Parkinsonism secondary to intravenous injection of meperidine analogues. Psychiatry Res 1: 249-254

Davis KL, Edin HM and Allen JK (2010) Prevalence and cost of medication nonadherence in Parkinson's disease: evidence from administrative claims data. Mov Disord. 2010 Mar 15;25(4):474-80

Dekundy A, Lundblad M, Danysz W, and Cenci MA (2007) Modulation of L-DOPA-induced abnormal involuntary movements by clinically tested compounds: further validation of the rat dyskinesia model. Behav Brain Res 179: 76-89

Del Amo EM and Urtti A (2008) Current and future ophthalmic drug delivery systems. A shift to the posterior segment. Drug Discov Today 13: 135-143

Del Amo EM, Heikkinen AT, Mönkkönen J (2009) In vitro-in vivo correlation in P-glycoprotein mediated transport in intestinal absorption. Eur J Pharm Sci. 2009 Feb 15;36(2-3):200-11

Dewey RB, Jr. (2004) Management of motor complications in Parkinson's disease. Neurology 62: S3-S7

Dexter DT and Jenner P (2013) Parkinson disease: from pathology to molecular disease mechanisms. Free Radic Biol Med

Di Monte DA, McCormack A, Petzinger G, Janson AM, Quik M, and Langston WJ (2000) Relationship among nigrostriatal denervation, parkinsonism, and dyskinesias in the MPTP primate model. Mov Disord 15: 459-466

Di SA, Sozio P, Cerasa LS, and Iannitelli A (2011) L-Dopa prodrugs: an overview of trends for improving Parkinson's disease treatment. Curr Pharm Des 17: 3482-3493

Di Stefano A, Sozio P, Cocco A, Iannitelli A, Santucci E, Costa M, Pecci L, Nasuti C, Cantalamessa F and Pinnen F (2006) L-dopa- and dopamine-(R)-alpha-lipoic acid conjugates as multifunctional codrugs with antioxidant properties. J Med Chem. 2006 Feb 23;49(4):1486-93

Di Stefano A, Sozio P and Cerasa LS (2008) Antiparkinson prodrugs. Molecules. 2008 Jan 16;13(1):46-68

Diamond SG and Markham CH (1984) One-year trial of pergolide as an adjunct to Sinemet in treatment of Parkinson's disease. Adv Neurol 40: 537-539

Dingemanse J, Kleinbloesem CH, Zurcher G, Wood ND, and Crevoisier C (1997a) Pharmacodynamics of benserazide assessed by its effects on endogenous and exogenous levodopa pharmacokinetics. Br J Clin Pharmacol 44: 41-48

Dingemanse J, Wood N, Jorga K, and Kettler R (1997b) Pharmacokinetics and pharmacodynamics of single and multiple doses of the MAO-B inhibitor lazabemide in healthy subjects. Br J Clin Pharmacol 43: 41-47

Djaldetti R, Inzelberg R, Giladi N, Korczyn AD, Peretz-Aharon Y, Rabey MJ, Herishano Y, Honigman S, Badarny S and Melamed E (2002) Oral solution of levodopa ethylester for treatment of response fluctuations in patients with advanced Parkinson's disease. Mov Disord. 2002 Mar;17(2):297-302

Doty RL (2012) Olfactory dysfunction in Parkinson disease. Nat Rev Neurol. 2012 May 15;8(6):329-39

Durso R, Evans JE, Josephs E, Szabo G, Evans B, Fernandez HH, and Browne TR (2000) Variable absorption of carbidopa affects both peripheral and central levodopa metabolism. J Clin Pharmacol 40: 854-860

Duty S and Jenner P (2011) Animal models of Parkinson's disease: a source of novel treatments and clues to the cause of the disease. Br J Pharmacol 164: 1357-1391

Droździk M, Białecka M, Kurzawski M (2013) Pharmacogenetics of Parkinson's disease - through mechanisms of drug actions. Curr Genomics. 2013 Dec;14(8):568-77.

Ekstrand MI and Galter D (2009) The MitoPark Mouse - an animal model of Parkinson's disease with impaired respiratory chain function in dopamine neurons. Parkinsonism Relat Disord. 2009 Dec;15 Suppl 3:S185-8

ELLIOTT H and HOOPER GD (1954) The shaking palsy and James Parkinson; the original description in 1817 by one of John Hunter's pupils. Treat Serv Bull 9: 115-120

Elm JJ, Kamp C, Tilley BC, Guimaraes P, Fraser D, Deppen P, Brocht A, Weaver C, Bennett S; NINDS NET-Parkinson's disease Investigators and Coordinators (2007) Self-reported adherence versus pill count in Parkinson's disease: the NET-Parkinson's disease experience. Mov Disord. 2007 Apr 30;22(6):822-7

Emborg ME (2007) Nonhuman primate models of Parkinson's disease. ILAR J 48: 339-355

Ekstrand MI, Galter D (2009) The MitoPark Mouse - an animal model of Parkinson's disease with impaired respiratory chain function in dopamine neurons. Parkinsonism Relat Disord. 2009 Dec;15 Suppl 3:S185-8

Eslamboli A (2005) Marmoset monkey models of Parkinson's disease: which model, when and why? Brain Res Bull 68: 140-149

Eslamboli A (2005) Marmoset monkey models of Parkinson's disease: which model, when and why? Brain Res Bull. 2005 Dec 30;68(3):140-9

Eskow Jaunarajs KL, Dupre KB, Ostock CY, Button T, Deak T, Bishop C (2010) Behavioral and neurochemical effects of chronic L-DOPA treatment on nonmotor sequelae in the hemiparkinsonian rat. Behav Pharmacol. 2010 Oct;21(7):627-37

Espinoza S, Manago F, Leo D, Sotnikova TD, Gainetdinov RR (2012) Role of catechol-O-methyltransferase (COMT)-dependent processes in Parkinson's disease and L-DOPA treatment. CNS Neurol Disord Drug Targets. 2012 May;11(3):251-63.

Felix AM, Winter DP, Wang SS, Kulesha ID, Pool WR, Hane DL and Sheppard H (1974) Synthesis and antireserpine activity of peptides of L-dopa. J Med Chem. 1974 Apr;17(4):422-6

Fiskum G, Murphy AN, and Beal MF (1999) Mitochondria in neurodegeneration: acute ischemia and chronic neurodegenerative diseases. J Cereb Blood Flow Metab 19: 351-369

Fornai F, Schlüter OM, Lenzi P, Gesi M, Ruffoli R, Ferrucci M, Lazzeri G, Busceti CL, Pontarelli F, Battaglia G, Pellegrini A, Nicoletti F, Ruggieri S, Paparelli A, Südhof TC (2005) Parkinson-like syndrome induced by continuous

MPTP infusion: convergent roles of the ubiquitin-proteasome system and alpha-synuclein. Proc Natl Acad Sci U S A. 2005 Mar 1;102(9):3413-8.

Foster HD and Hoffer A (2004) The two faces of L-DOPA: benefits and adverse side effects in the treatment of Encephalitis lethargica, Parkinson's disease, multiple sclerosis and amyotrophic lateral sclerosis. Med Hypotheses 62: 177-181

Fox SH, Henry B, Hill M, Crossman A, and Brotchie J (2002) Stimulation of cannabinoid receptors reduces levodopa-induced dyskinesia in the MPTP-lesioned nonhuman primate model of Parkinson's disease. Mov Disord 17: 1180-1187

Galvez-Jimenez N, Hanson MR, and Cabral J (2000) Dopa-resistant parkinsonism, oculomotor disturbances, chorea, mirror movements, dyspraxia, and dementia: the expanding clinical spectrum of hypoparathyroidism. A case report. Mov Disord 15: 1273-1276

Gasser UE, Jorga K, Crevoisier C, Hovens SE, and van Giersbergen PL (1999) COMT inhibition by tolcapone further improves levodopa pharmacokinetics when combined with a dual-release formulation of levodopa/benserazide. A novel principle in the treatment of Parkinson's disease. Eur Neurol 41: 206-211

Gaytan O, Swann A, and Dafny N (1998a) Diurnal differences in rat's motor response to amphetamine. Eur J Pharmacol 345: 119-128

Gaytan O, Swann A, and Dafny N (1998b) Time-dependent differences in the rat's motor response to amphetamine. Pharmacol Biochem Behav 59: 459-467

Gerfen CR (2000) Molecular effects of dopamine on striatal-projection pathways. Trends Neurosci. 2000 Oct;23(10 Suppl):S64-70.

Goetz CG, Poewe W, Rascol O, and Sampaio C (2005a) Evidence-based medical review update: pharmacological and surgical treatments of Parkinson's disease: 2001 to 2004. Mov Disord 20: 523-539

Goetz CG, Wuu J, Curgian LM, and Leurgans S (2005b) Hallucinations and sleep disorders in Parkinson's disease: six-year prospective longitudinal study. Neurology 64: 81-86

Grange S, Holford NH, and Guentert TW (2001) A pharmacokinetic model to predict the PK interaction of L-dopa and benserazide in rats. Pharm Res 18: 1174-1184

Gravotta L, Gavrila AM, Hood S, and Amir S (2011) Global depletion of dopamine using intracerebroventricular 6-hydroxydopamine injection disrupts normal circadian wheel-running patterns and PERIOD2 expression in the rat forebrain. J Mol Neurosci 45: 162-171

Greenacre JK, Coxon A, Petrie A, and Reid JL (1976) Comparison of levodopa with carbidopa or benserazide in parkinsonism. Lancet 2: 381-384

Haapalinna A, Leino T, and Heinonen E (2003) The alpha 2-adrenoceptor antagonist atipamezole potentiates anti-Parkinsonian effects and can reduce the adverse cardiovascular effects of dopaminergic drugs in rats. Naunyn Schmiedebergs Arch Pharmacol 368: 342-351

Hadjiconstantinou M and Neff NH (2008) Enhancing aromatic L-amino acid decarboxylase activity: implications for L-DOPA treatment in Parkinson's disease. CNS Neurosci Ther 14: 340-351

Hagan RM, Raxworthy MJ, and Gulliver PA (1980) Benserazide and carbidopa as substrates of catechol-O-methyltransferase: new mechanism of action in Parkinson's disease. Biochem Pharmacol 29: 3123-3126

Hallett PJ, Dunah AW, Ravenscroft P, Zhou S, Bezard E, Crossman AR, Brotchie JM, Standaert DG (2005) Alterations of striatal NMDA receptor subunits associated with the development of dyskinesia in the MPTP-lesioned primate model of Parkinson's disease. Neuropharmacology. 2005 Mar;48(4):503-16.

Hallett M (2012) Parkinson's disease tremor: pathophysiology. Parkinsonism Relat Disord. 2012 Jan;18 Suppl 1:S85-6

Halliday GM, Blumbergs PC, Cotton RG, Blessing WW, and Geffen LB (1990) Loss of brainstem serotonin- and substance P-containing neurons in Parkinson's disease. Brain Res 510: 104-107

Hanson MR and Galvez-Jimenez N (2001) Catechol-O-methyltransferase inhibitors in the management of Parkinson's disease. Semin Neurol 21: 15-22

Hashimoto T, Tada T, Nakazato F (2001) Abnormal activity in the globus pallidus in off-period dystonia. Ann. Neurol. 49(2), 242–245

Hauber W, Lutz S, Münkle M (1998) The effects of globus pallidus lesions on dopamine-dependent motor behaviour in rats. Neuroscience.

Hauser RA, Hubble JP, and Truong DD (2003) Randomized trial of the adenosine A(2A) receptor antagonist istradefylline in advanced Parkinson's disease. Neurology 61: 297-303

Henry B, Crossman AR, and Brotchie JM (1998) Characterization of enhanced behavioral responses to L-DOPA following repeated administration in the 6-hydroxydopamine-lesioned rat model of Parkinson's disease. Exp Neurol 151: 334-342

Hauber W (1998) Blockade of subthalamic dopamine D1 receptors elicits akinesia in rats. Neuroreport Dec 21;9(18):4115-8.

Hermida-Ameijeiras A, Mendez-Alvarez E, Sanchez-Iglesias S, Sanmartin-Suarez C, and Soto-Otero R (2004) Autoxidation and MAO-mediated metabolism of dopamine as a potential cause of oxidative stress: role of ferrous and ferric ions. Neurochem Int 45: 103-116

Herrero MT, Pagonabarraga J, Linazasoro G (2011) Neuroprotective role of dopamine agonists: evidence from animal models and clinical studies. Neurologist. 2011 Nov;17(6 Suppl 1):S54-66

Hironishi M, Miwa H, Kondo T (2002) Benefit of L-DOPA-without-DCI (decarboxylase inhibitor) therapy on wearing-off phenomenon in advanced stages of Parkinson's disease patients. No To Shinkei. 2002 Feb;54(2):127-32

HESS R (1961) [The electronecephalogram in Parkinson's syndrome, before and after stereotactic surgery]. Schweiz Arch Neurol Neurochir Psychiatr 87: 322-324

Hisaka A, Kasamatsu S, Takenaga N, and Ohtawa M (1989) Quantification of L-3-(3-hydroxy-4-pivaloyloxyphenyl)alanine (NB-355) by high-performance liquid chromatography using o-phthalaldehyde/N-acetyl-L-cysteine derivatization. J Chromatogr 494: 183-189

Hobson DE, Pourcher E, and Martin WR (1999) Ropinirole and pramipexole, the new agonists. Can J Neurol Sci 26 Suppl 2: S27-S33

HODGE JV, OATES JA, and SJOERDSMA A (1964) REDUCTION OF THE CENTRAL EFFECTS OF TRYPTOPHAN BY A DECARBOXYLASE INHIBITOR. Clin Pharmacol Ther 5: 149-155

HOLTZ P (1959) Role of L-DOPA decarboxylase in the biosynthesis of catecholamines in nervous tissue and the adrenal medulla. Pharmacol Rev 11: 317-329

HOLTZ P and WESTERMANN E (1956) [Nerve tissue DOPA decarboxylase and histidine decarboxylase]. Naunyn Schmiedebergs Arch Exp Pathol Pharmakol 227: 538-546

Honig H, Antonini A, Martinez-Martin P, Forgacs I, Faye GC, Fox T, Fox K, Mancini F, Canesi M, Odin P, and Chaudhuri KR (2009a) Intrajejunal levodopa infusion in Parkinson's disease: A pilot multicenter study of effects on nonmotor symptoms and quality of life. Mov Disord

Honig H, Antonini A, Martinez-Martin P, Forgacs I, Faye GC, Fox T, Fox K, Mancini F, Canesi M, Odin P, and Chaudhuri KR (2009b) Intrajejunal levodopa infusion in Parkinson's disease: a pilot multicenter study of effects on nonmotor symptoms and quality of life. Mov Disord 24: 1468-1474

Horne R, Weinman J (1999) Patients' beliefs about prescribed medicines and their role in adherence to treatment in chronic physical illness. J Psychosom Res. 1999 Dec;47(6):555-67

Hornykiewicz O (2001) Dopamine and Parkinson's disease. A personal view of the past, the present, and the future. Adv Neurol 86: 1-11

Horowski R (1988) Factors influencing dopaminergic therapy of Parkinson's disease. Funct Neurol 3: 459-470

Horowski R, Marsden CD, and Obeso JA (1988) Continuous dopaminergic stimulation: state of the art and outlook. J Neural Transm Suppl 27: 249-252

Hubble JP (2002) Long-term studies of dopamine agonists. Neurology 58: S42-S50

Hudson JL, van Horne CG, Stromberg I, Brock S, Clayton J, Masserano J, Hoffer BJ, and Gerhardt GA (1993) Correlation of apomorphine- and amphetamine-induced turning with nigrostriatal dopamine content in unilateral 6-hydroxydopamine lesioned rats. Brain Res 626: 167-174

Hutton JT and Morris JL (1991) Long-term evaluation of Sinemet CR in parkinsonian patients with motor fluctuations. Can J Neurol Sci. 1991 Nov;18(4):467-71

Ihara M, Tsuchiya Y, Sawasaki Y, Hisaka A, Takehana H, Tomimoto K, and Yano M (1989) A new potential prodrug to improve the duration of L-dopa: L-3-(3-hydroxy-4-pivaloyloxyphenyl)alanine. J Pharm Sci 78: 525-529

Iravani MM, Tayarani-Binazir K, Chu WB, Jackson MJ, and Jenner P (2006) In 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-treated primates, the selective 5-hydroxytryptamine 1a agonist (R)-(+)-8-OHDPAT inhibits

levodopa-induced dyskinesia but only with\ increased motor disability. J Pharmacol Exp Ther 319: 1225-1234

Ishikura N, Hamamoto M, Ohtsubo K, Miyazaki T (1995) A pharmacological study of levodopa solution therapy for a patient with Parkinson's disease with motor fluctuations. Nihon Ronen Igakkai Zasshi Jan;32(1):51-2.

Iwamoto K, Watanabe J, and Aoyama Y (1987a) High capacity for pulmonary first-pass elimination of propranolol in rats. J Pharm Pharmacol 39: 1049-1051

Iwamoto K, Watanabe J, and Atsumi F (1987b) Age dependence in capacity-limited jejunal decarboxylation of levodopa in rats. Biochem Pharmacol 36: 2151-2155

Iwamoto K, Watanabe J, Yamada M, Atsumi F, and Matsushita T (1987c) Effect of age on gastrointestinal and hepatic first-pass effects of levodopa in rats. J Pharm Pharmacol 39: 421-425

Jackson MJ, Al-Barghouthy G, Pearce RK, Smith L, Hagan JJ, and Jenner P (2004) Effect of 5-HT1B/D receptor agonist and antagonist administration on motor function in haloperidol and MPTP-treated common marmosets. Pharmacol Biochem Behav 79: 391-400

Jackson MJ, Smith LA, Al-Barghouthy G, Rose S, and Jenner P (2007) Decreased expression of l-dopa-induced dyskinesia by switching to

ropinirole in MPTP-treated common marmosets. Exp Neurol 204: 162-170

Jankovic J (2005) Motor fluctuations and dyskinesias in Parkinson's disease: clinical manifestations. Mov Disord 20 Suppl 11: S11-S16

Jeffcoate W (1999) Assessment of corticosteroid replacement therapy in adults with adrenal insufficiency. Ann Clin Biochem. 1999 Mar;36 (Pt 2):151-7

Jenner P (2000) Factors influencing the onset and persistence of dyskinesia in MPTP-treated primates. Ann Neurol 47: S90-S99

Jenner P (2002) Pharmacology of dopamine agonists in the treatment of Parkinson's disease. Neurology 58: S1-S8

Jenner P (2003) The contribution of the MPTP-treated primate model to the development of new treatment strategies for Parkinson's disease. Parkinsonism Relat Disord 9: 131-137

Jenner P (2004) Avoidance of dyskinesia: preclinical evidence for continuous dopaminergic stimulation. Neurology 62: S47-S55

Jenner P (2009) From the MPTP-treated primate to the treatment of motor complications in Parkinson's disease. Parkinsonism Relat Disord 15 Suppl 4: S18-S23

Jenner P and Marsden CD (1986) The actions of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in animals as a model of Parkinson's disease. J Neural Transm Suppl 20: 11-39

Jenner P, Rupniak NM, Rose S, Kelly E, Kilpatrick G, Lees A, and Marsden CD (1984) 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced parkinsonism in the common marmoset. Neurosci Lett 50: 85-90

Jenner P (2002) Pharmacology of dopamine agonists in the treatment of Parkinson's disease. Neurology. 2002 Feb 26;58(4 Suppl 1):S1-8.

Jenner PG and Brin MF (1998) Levodopa neurotoxicity: experimental studies versus clinical relevance. Neurology 50: S39-S43

Jenner P, McCreary AC, Scheller DK (2011) Continuous drug delivery in early- and late-stage Parkinson's disease as a strategy for avoiding dyskinesia induction and expression. J Neural Transm. 2011 Dec;118(12):1691-702

Jiang C, Wan X, Jankovic J, Christian ST, Pristupa ZB, Niznik HB, Sundsmo JS, Le W (2004) Dopaminergic properties and experimental anti-parkinsonian effects of IPX750 in rodent models of Parkinson disease. Clin Neuropharmacol. 2004 Mar-Apr;27(2):63-73

Jonkers N, Sarre S, Ebinger G, and Michotte Y (2001) Benserazide decreases central AADC activity, extracellular dopamine levels and levodopa decarboxylation in striatum of the rat. J Neural Transm 108: 559-570

Juncos JL, Mouradian MM, Fabbrini G, Serrati C and Chase TN (1987) Levodopa methyl ester treatment of Parkinson's disease. Neurology. 1987 Jul;37(7):1242-5

Kaakkola S and Teravainen H (1990) Animal models of parkinsonism. Pharmacol Toxicol 67: 95-100

Kanda T, Jackson MJ, Smith LA, Pearce RK, Nakamura J, Kase H, Kuwana Y, and Jenner P (1998a) Adenosine A2A antagonist: a novel antiparkinsonian agent that does not provoke dyskinesia in parkinsonian monkeys. Ann Neurol 43: 507-513

Kanda T, Tashiro T, Kuwana Y, and Jenner P (1998b) Adenosine A2A receptors modify motor function in MPTP-treated common marmosets. Neuroreport 9: 2857-2860

Kase H, Aoyama S, Ichimura M, Ikeda K, Ishii A, Kanda T, Koga K, Koike N, Kurokawa M, Kuwana Y, Mori A, Nakamura J, Nonaka H, Ochi M, Saki M, Shimada J, Shindou T, Shiozaki S, Suzuki F, Takeda M, Yanagawa K, Richardson PJ, Jenner P, Bedard P, Borrelli E, Hauser RA, Chase TN; KW-6002 US-001 Study Group (2003) Progress in pursuit of therapeutic A2A antagonists: the adenosine A2A receptor selective antagonist KW6002: research and development toward a novel nondopaminergic therapy for Parkinson's disease. Neurology. 2003 Dec 9;61(11 Suppl 6):S97-100

Katzenschlager R, Sampaio C, Costa J, and Lees A (2003) Anticholinergics for symptomatic management of Parkinson's disease. Cochrane Database Syst RevCD003735

Khan NL, Graham E, Critchley P, Schrag AE, Wood NW, Lees AJ, Bhatia KP, and Quinn N (2003) Parkin disease: a phenotypic study of a large case series. Brain 126: 1279-1292

Kish SJ, Shannak K, and Hornykiewicz O (1988) Uneven pattern of dopamine loss in the striatum of patients with idiopathic Parkinson's disease. Pathophysiologic and clinical implications. N Engl J Med 318: 876-880

Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, Yokochi M, Mizuno Y, and Shimizu N (1998) Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. Nature 392: 605-608

Kocabicak E and Temel Y (2013) Deep brain stimulation of the subthalamic nucleus in Parkinson's disease: Surgical technique, tips, tricks and complications. Clin Neurol Neurosurg. 2013 Nov;115(11):2318-23

Koller WC (2002) Treatment of early Parkinson's disease. Neurology 58: S79-S86

Koller WC, Pahwa R (1994) Treating motor fluctuations with controlled-release levodopa preparations. Neurology. 1994 Jul;44(7 Suppl 6):S23-8

Korten JJ, Keyser A, Joosten EM, and Gabreels FJ (1975) Madopar versus sinemet. A clinical study on their effectiveness. Eur Neurol 13: 65-71

Kulisevsky J, Barbanoj M, Gironell A, Antonijoan R, Casas M, and Pascual-Sedano B (2002) A double-blind crossover, placebo-controlled study of the adenosine A2A antagonist theophylline in Parkinson's disease. Clin Neuropharmacol 25: 25-31

Kuoppamaki M, Korpela K, Marttila R, Kaasinen V, Hartikainen P, Lyytinen J, Kaakkola S, Hanninen J, Loyttyniemi E, Kailajarvi M, Ruokoniemi P, and Ellmen J (2009) Comparison of pharmacokinetic profile of levodopa throughout the day between levodopa/carbidopa/entacapone and levodopa/carbidopa when administered four or five times daily. Eur J Clin Pharmacol 65: 443-455

Kuwana Y, Shiozaki S, Kanda T, Kurokawa M, Koga K, Ochi M, Ikeda K, Kase H, Jackson MJ, Smith LA, Pearce RK, and Jenner PG (1999) Antiparkinsonian activity of adenosine A2A antagonists in experimental models. Adv Neurol 80: 121-123

Kyriazis M (2003) Neuroprotective, anti-apoptotic effects of apomorphine. J Anti Aging Med 6: 21-28

Lane EL, Cheetham S, and Jenner P (2008) Striatal output markers do not alter in response to circling behaviour in 6-OHDA lesioned rats produced by acute or chronic administration of the monoamine uptake inhibitor BTS 74 398. J Neural Transm 115: 423-429

Lane EL, Cheetham SC, Jenner P (2006) Does contraversive circling in the 6-OHDA-lesioned rat indicate an ability to induce motor complications as well as therapeutic effects in Parkinson's disease? Exp Neurol. 2006 Feb;197(2):284-90.

Langston JW (1985) MPTP neurotoxicity: an overview and characterization of phases of toxicity. Life Sci 36: 201-206

Langston JW, Irwin I (1986) MPTP: current concepts and controversies. Clin Neuropharmacol. 9(6):485-507. Review

Langston JW (1990) Predicting Parkinson's disease. Neurology 40: suppl-4

Langston JW, Ballard P, Tetrud JW, and Irwin I (1983) Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. Science 219: 979-980

Lee KE, Choi YJ, Oh BR, Chun IK, Gwak HS (2013) Formulation and in vitro/in vivo evaluation of levodopa transdermal delivery systems. Int J Pharm. 2013 Nov 18;456(2):432-6

Lee YH, Kim KH, Yoon IK, Lee KE, Chun IK, Rhie JY, Gwak HS (2013) Pharmacokinetic evaluation of formulated levodopa methyl ester nasal delivery systems. Eur J Drug Metab Pharmacokinet. Dec 21

Lees AJ, Katzenschlager R, Head J, and Ben-Shlomo Y (2001) Ten-year follow-up of three different initial treatments in de-novo Parkinson's disease: a randomized trial. Neurology 57: 1687-1694

Leppert PS, Cortese M, and Fix JA (1988) The effects of carbidopa dose and time and route of administration on systemic L-dopa levels in rats. Pharm Res 5: 587-591

Leroy E, Anastasopoulos D, Konitsiotis S, Lavedan C, and Polymeropoulos MH (1998) Deletions in the Parkin gene and genetic heterogeneity in a Greek family with early onset Parkinson's disease. Hum Genet 103: 424-427

Lewis B, O'Donnell P (2003) Blockade of the GlyT1 glycine transporter prolongs response to VTA stimulation in nucleus accumbens neurons. Ann N Y Acad Sci. 2003 Nov;1003:431-4

Lieberman A, Estey E, Gopinathan G, Ohashi T, Sauter A, and Goldstein M (1978) Comparative effectiveness of two extracerebral DOPA decarboxylase inhibitors in Parkinson disease. Neurology 28: 964-968

Lieberman A, Goodgold A, Jonas S, and Leibowitz M (1975) Comparison of dopa decarboxylase inhibitor (carbidopa) combined with levodopa and levodopa alone in Parkinson's disease. Neurology 25: 911-916

Lundblad M, Usiello A, Carta M, Håkansson K, Fisone G and Cenci MA (2005) Pharmacological validation of a mouse model of l-DOPA-induced dyskinesia. Exp Neurol. 2005 Jul;194(1):66-75

Magariños-Ascone C, Pazo JH, Macadar O, Buño W (2002) High-frequency stimulation of the subthalamic nucleus silences subthalamic neurons: a possible cellular mechanism in Parkinson's disease. Neuroscience. 2002;115(4):1109-17

Magnussen I, Jensen TS, Rand JH, and Van Woert MH (1981) Plasma accumulation of metabolism of orally administered single dose L-5-hydroxytryptophan in man. Acta Pharmacol Toxicol (Copenh) 49: 184-189

Magyar K and Szende B (2004) (-)-Deprenyl, a selective MAO-B inhibitor, with apoptotic and anti-apoptotic properties. Neurotoxicology 25: 233-242

Mah GT, Tejani AM, Musini VM (2009) Methyldopa for primary hypertension. Cochrane Database Syst Rev. 2009 Oct 7;(4):CD003893

Manson A, Stirpe P, Schrag A (2012) Levodopa-induced-dyskinesias clinical features, incidence, risk factors, management and impact on quality of life. J Parkinsons Dis. 2012;2(3):189-98.

Maratos EC, Jackson MJ, Pearce RK, and Jenner P (2001) Antiparkinsonian activity and dyskinesia risk of ropinirole and L-DOPA combination therapy in drug naive MPTP-lesioned common marmosets (Callithrix jacchus). Mov Disord 16: 631-641

Marien MR, Colpaert FC, and Rosenquist AC (2004) Noradrenergic mechanisms in neurodegenerative diseases: a theory. Brain Res Brain Res Rev 45: 38-78

Marin C, Aguilar E, Mengod G, Cortés R, Obeso JA (2009) Effects of early vs. late initiation of levodopa treatment in hemiparkinsonian rats. Eur J Neurosci. 2009 Sep;30(5):823-32

Marsden CD and Olanow CW (1999) The causes of Parkinson's disease are being unraveled and rational neuroprotective therapy is close to reality. Ann Neurol. 1998 Sep;44(3 Suppl 1):S189-96

Marsden CA and Guldberg HC (1973a) The role of monoamines in rotation induced or potentiated by amphetamine after nigral, raphe and mesencephalic reticular lesions in the rat brain. Neuropharmacology 12: 195-211

Marsden CD, Barry PE, Parkes JD, and Zilkha KJ (1973b) Treatment of Parkinson's disease with levodopa combined with L-alpha-methyldopahydrazine, an inhibitor of extracerebral DOPA decarboxylase. J Neurol Neurosurg Psychiatry 36: 10-14

Marsden CD, Parkes JD, and Rees JE (1973c) A year's comparison of treatment of patients with parkinson's disease with levodopa combined with carbidopa versus treatment with levodopa alone. Lancet 2: 1459-1462

Mao Z, Hsu A, Gupta S, Modi NB (2013) Population pharmacodynamics of IPX066: an oral extended-release capsule formulation of carbidopa-levodopa, and immediate-release carbidopa-levodopa in patients with advanced Parkinson's disease. J Clin Pharmacol. 2013 May;53(5):523-31

McColl CD, Reardon KA, Shiff M, and Kempster PA (2002) Motor response to levodopa and the evolution of motor fluctuations in the first decade of treatment of Parkinson's disease. Mov Disord 17: 1227-1234

McAuley JH (2003) The physiological basis of clinical deficits in Parkinson's disease. Prog Neurobiol. 2003 Jan;69(1):27-48.

McNaught KS and Jenner P (2001a) Proteasomal function is impaired in substantia nigra in Parkinson's disease. Neurosci Lett 297: 191-194

McNaught KS and Olanow CW (2006) Protein aggregation in the pathogenesis of familial and sporadic Parkinson's disease. Neurobiol Aging 27: 530-545

McNaught KS, Olanow CW, Halliwell B, Isacson O, and Jenner P (2001b) Failure of the ubiquitin-proteasome system in Parkinson's disease. Nat Rev Neurosci 2: 589-594

McNaught KS, Perl DP, Brownell AL, and Olanow CW (2004) Systemic exposure to proteasome inhibitors causes a progressive model of Parkinson's disease. Ann Neurol 56: 149-162

Mitchell IJ, Clarke CE, Boyce S, Robertson RG, Peggs D, Sambrook MA, Crossman AR (1989) Neural mechanisms underlying parkinsonian symptoms based upon regional uptake of 2-deoxyglucose in monkeys exposed to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Neuroscience 32(1):213-26.

Montastruc JL, Rascol O, and Senard JM (1999) Treatment of Parkinson's disease should begin with a dopamine agonist. Mov Disord 14: 725-730

Monville C, Torres EM, Pekarik V, Lane EL, and Dunnett SB (2009) Genetic, temporal and diurnal influences on L-dopa-induced dyskinesia in the 6-OHDA model. Brain Res Bull 78: 248-253

Morin N, Jourdain VA and Di Paolo T (2013) Modeling dyskinesia in animal models of Parkinson disease. Exp Neurol. 2013 Jan 27. doi:pii: S0014-4886(13)

Mura A, Mintz M, and Feldon J (2002) Behavioral and anatomical effects of long-term L-dihydroxyphenylalanine (L-DOPA) administration in rats with unilateral lesions of the nigrostriatal system. Exp Neurol 177: 252-264

Muralikrishnan D, Samantaray S, and Mohanakumar KP (2003) D-deprenyl protects nigrostriatal neurons against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced dopaminergic neurotoxicity. Synapse 50: 7-13

Morelli M, Carta AR, Jenner P (2009) Adenosine A2A receptors and Parkinson's disease. Handb Exp Pharmacol. 2009;(193):589-615.

Neff NH and Yang HY (1974) Another look at the monoamine oxidases and the monoamine oxidase inhibitor drugs. Life Sci 14: 2061-2074

Nishimura K, Takahashi J (2013) Therapeutic application of stem cell technology toward the treatment of Parkinson's disease. Biol Pharm Bull. 36(2):171-5

Nissinen H, Kuoppamäki M, Leinonen M, Schapira AH (2009) Early versus delayed initiation of entacapone in levodopa-treated patients with Parkinson's disease: a long-term, retrospective analysis. Eur J Neurol. 2009 Dec;16(12):1305-11

Ngwuluka N (2010) Application of in situ polymerization for design and development of oral drug delivery systems. AAPS PharmSciTech 11: 1603-1611

Ngwuluka N, Pillay V, Du Toit LC, Ndesendo V, Choonara Y, Modi G, and Naidoo D (2010) Levodopa delivery systems: advancements in delivery of the gold standard. Expert Opin Drug Deliv 7: 203-224

Nomoto M, Kaseda S, Iwata S, Shimizu T, Fukuda T, and Nakagawa S (2000) The metabolic rate and vulnerability of dopaminergic neurons, and adenosine dynamics in the cerebral cortex, nucleus accumbens, caudate

nucleus, and putamen of the common marmoset. J Neurol 247 Suppl 5: V16-V22

Nordera GP, Lorizio A, Lion P, Durisotti C, D'Andrea G, Ferro-Milone F (1987) Treatment of parkinsonian conditions with a controlled-release form of levodopa--preliminary study. Eur Neurol. 1987;27 Suppl 1:76-80.

Nutt JG (2008) Pharmacokinetics and pharmacodynamics of levodopa. Mov Disord 23 Suppl 3: S580-S584

Nutt JG and Woodward WR (1986a) Levodopa pharmacokinetics and pharmacodynamics in fluctuating parkinsonian patients. Neurology 36: 739-744

Nutt JG, Woodward WR, and Anderson JL (1985) The effect of carbidopa on the pharmacokinetics of intravenously administered levodopa: the mechanism of action in the treatment of parkinsonism. Ann Neurol 18: 537-543

Nutt JG, Woodward WR, and Carter JH (1986b) Clinical and biochemical studies with controlled-release levodopa/carbidopa. Neurology 36: 1206-1211

Nutt JG, Woodward WR, Hammerstad JP, Carter JH, and Anderson JL (1984) The "on-off" phenomenon in Parkinson's disease. Relation to levodopa absorption and transport. N Engl J Med 310: 483-488

Obeso JA, Luquin MR, Vaamonde J, Grandas F, and Martinez Lage JM (1987) Continuous dopaminergic stimulation in Parkinson's disease. Can J Neurol Sci 14: 488-492

Obeso JA, Olanow CW, and Nutt JG (2000a) Levodopa motor complications in Parkinson's disease. Trends Neurosci 23: S2-S7

Obeso JA, Rodriguez-Oroz MC, Chana P, Lera G, Rodriguez M, and Olanow CW (2000b) The evolution and origin of motor complications in Parkinson's disease. Neurology 55: S13-S20

Obeso JA, Rodriguez-Oroz MC, Rodriguez M, DeLong MR, and Olanow CW (2000c) Pathophysiology of levodopa-induced dyskinesias in Parkinson's disease: problems with the current model. Ann Neurol 47: S22-S32

Obeso JA, Rodriguez-Oroz MC, Rodriguez M, Lanciego JL, Artieda J, Gonzalo N, and Olanow CW (2000d) Pathophysiology of the basal ganglia in Parkinson's disease. Trends Neurosci 23: S8-19

Obeso JA, Rodriguez-Oroz MC, Chana P, Lera G, Rodriguez M, Olanow CW (2000) The evolution and origin of motor complications in Parkinson's disease. Neurology. 55(11 Suppl 4):S13-20.

Oertel WH, Benes H, Bodenschatz R, Peglau I, Warmuth R, Happe S, Geisler P, Cassel W, Leroux M, Kohnen R, and Stiasny-Kolster K (2006) Efficacy of cabergoline in restless legs syndrome: a placebo-controlled study with polysomnography (CATOR). Neurology 67: 1040-1046

Ohtsuki S and Terasaki T (2007) Contribution of carrier-mediated transport systems to the blood-brain barrier as a supporting and protecting interface for the brain; importance for CNS drug discovery and development. Pharm Res 24: 1745-1758

Olanow CW (2002) The role of dopamine agonists in the treatment of early Parkinson's disease. Neurology 58: S33-S41

Olanow CW and Stocchi F (2000) Why delaying levodopa is a good treatment strategy in early Parkinson's disease. Eur J Neurol 7 Suppl 1: 3-8

Olanow CW and Tatton WG (1999) Etiology and pathogenesis of Parkinson's disease. Annu Rev Neurosci 22: 123-144

Olanow CW, Schapira AH (2013) Therapeutic prospects for Parkinson disease. Ann Neurol. 2013 Sep;74(3):337-47

Oldendorf WH, Hyman S, Braun L, and Oldendorf SZ (1972) Blood-brain barrier: penetration of morphine, codeine, heroin, and methadone after carotid injection. Science 178: 984-986

Paisan-Ruiz C, Jain S, Evans EW, Gilks WP, Simon J, van der BM, Lopez de MA, Aparicio S, Gil AM, Khan N, Johnson J, Martinez JR, Nicholl D, Carrera IM, Pena AS, de SR, Lees A, Marti-Masso JF, Perez-Tur J, Wood NW, and Singleton AB (2004) Cloning of the gene containing mutations that cause PARK8-linked Parkinson's disease. Neuron 44: 595-600

Palm D, Ernzerhoff C, and HOLTZ P (1967) [On the activation of dopa decarboxylase and other cytoplasmic liver enzymes by inducers of microsomal enzymes]. Naunyn Schmiedebergs Arch Exp Pathol Pharmakol 258: 352-371

Papa SM, Engber TM, Kask AM, and Chase TN (1994) Motor fluctuations in levodopa treated parkinsonian rats: relation to lesion extent and treatment duration. Brain Res 662: 69-74

Paradiso G, Saint-Cyr JA, Lozano AM, Lang AE, Chen R (2003) Involvement of the human subthalamic nucleus in movement preparation. Neurology. Dec 9;61(11):1538-45

Park S, Kim E, Koh SE, Maeng S, Lee WD, Lim J, Shim I, Lee YJ (2012) Dopaminergic differentiation of neural progenitors derived from placental mesenchymal stem cells in the brains of Parkinson's disease model rats and alleviation of asymmetric rotational behavior. Brain Res. 2012 Jul 23;1466:158-66

Paxinos G, Watson C, Pennisi M, and Topple A (1985) Bregma, lambda and the interaural midpoint in stereotaxic surgery with rats of different sex, strain and weight. J Neurosci Methods 13: 139-143

Pearce RK, Banerji T, Jenner P, and Marsden CD (1998) De novo administration of ropinirole and bromocriptine induces less dyskinesia than L-dopa in the MPTP-treated marmoset. Mov Disord 13: 234-241

Pearce RK, Jackson M, Smith L, Jenner P, and Marsden CD (1995) Chronic L-DOPA administration induces dyskinesias in the 1-methyl-4- phenyl-1,2,3,6-tetrahydropyridine-treated common marmoset (Callithrix Jacchus). Mov Disord 10: 731-740

Pezzoli G, Tesei S, Cossutta E, Ferrante C, Zecchinelli A, Scarlato G (1988) [An open study of a new formulation of the levodopa and benserazide combination in the treatment of fluctuations of motor performance in patients with Parkinson's disease]. Clin Ter. 1988

Phani S, Loike JD, Przedborski S (2012) Neurodegeneration and inflammation in Parkinson's disease. Parkinsonism Relat Disord. Jan;18 Suppl 1:S207-9.

Phillipson OT (2013) Inhibition of aging in Parkinson's disease: a case study. J Altern Complement Med. Oct;19(10):851.

Picconi B, Centonze D, Håkansson K, Bernardi G, Greengard P, Fisone G, Cenci MA, Calabresi P (2003) Loss of bidirectional striatal synaptic plasticity in L-DOPA-induced dyskinesia. Nat Neurosci. 2003 May;6(5):501-6

Picconi B, Paille V, Ghiglieri V, Bagetta V, Barone I, Lindgren HS, Bernardi G, Angela CM, and Calabresi P (2008) l-DOPA dosage is critically involved in dyskinesia via loss of synaptic depotentiation. Neurobiol Dis 29: 327-335

Pilleri M, Antonini A (2014) Novel levodopa formulations in the treatment of Parkinson's disease. Expert Rev Neurother. 2014 Feb;14(2):143-9

Pinder RM, Brogden RN, Sawyer PR, Speight TM, and Avery GS (1976) Levodopa and decarboxylase inhibitors: a review of their clinical pharmacology and use in the treatment of parkinsonism. Drugs 11: 329-377

Pisani A, Bonsi P, Catania MV, Giuffrida R, Morari M, Marti M, Centonze D, Bernardi G, Kingston AE, and Calabresi P (2002) Metabotropic glutamate 2 receptors modulate synaptic inputs and calcium signals in striatal cholinergic interneurons. J Neurosci 22: 6176-6185

PLETSCHER A and GEY KF (1963) The effect of a new decarboxylase inhibitor on endogenous monoamines. Biochem Pharmacol 12: 223-228

Pollack AE, Harrison MB, Wooten GF, Fink JS (1993) Differential localization of A2a adenosine receptor mRNA with D1 and D2 dopamine receptor mRNA in striatal output pathways following a selective lesion of striatonigral neurons. Brain Res. 1993 Dec 17;631(1):161-6.

PORTER CC, TOTARO JA, and Stone CA (1963) Effect of 6-hydroxydopamine and some other compounds on the concentration of norepinephrine in the hearts of mice. J Pharmacol Exp Ther 140: 308-316

Potts LF, Wu H, Singh A, Marcilla I, Luquin MR, Papa SM (2013) Modeling Parkinson's disease in monkeys for translational studies, a critical analysis. Exp Neurol. 2013 Sep 23. pii: S0014-4886(13)00282-3

Quik M, Police S, Langston JW, and Di Monte DA (2002) Increases in striatal preproenkephalin gene expression are associated with nigrostriatal damage but not L-DOPA-induced dyskinesias in the squirrel monkey. Neuroscience 113: 213-220

Rascol O, Brooks DJ, Korczyn AD, De Deyn PP, Clarke CE, and Lang AE (2000a) A five-year study of the incidence of dyskinesia in patients with early Parkinson's disease who were treated with ropinirole or levodopa. 056 Study Group. N Engl J Med 342: 1484-1491

Rascol O and Montastruc JL (2000b) [Role of dopaminergic agonists]. Rev Neurol (Paris) 156 Suppl 2 Pt 2: 98-104

Rascol O, Payoux P, Ferreira J, and Brefel-Courbon C (2002) The management of patients with early Parkinson's disease. Parkinsonism Relat Disord 9: 61-67

Rascol O, Ferreira J, Nègre-Pages L, Perez-Lloret S, Lacomblez L, Galitzky M, Lemarié JC, Corvol JC, Brotchie JM, Bossi L (2012) A proof-of-concept, randomized, placebo-controlled, multiple cross-overs (n-of-1) study of naftazone in Parkinson's disease. Fundam Clin Pharmacol. 2012 Aug;26(4):557-64.

Rauws AG, Vos JG, Garbis-Berkvens JM, Peters PW, de VT, and van Logten MJ (1982) Comparative 90-day toxicity of two decarboxylase inhibitors, benserazide and carbidopa, in the rat. Toxicol Appl Pharmacol 66: 201-220

Rouse ST, Marino MJ, Bradley SR, Awad H, Wittmann M, Conn PJ (2000) Distribution and roles of metabotropic glutamate receptors in the basal ganglia motor circuit: implications for treatment of Parkinson's disease and related disorders. Pharmacol Ther Dec;88(3):427-35.

Reches A and Fahn S (1982) 3-O-methyldopa blocks dopa metabolism in rat corpus striatum. Ann Neurol 12: 267-271

Reid JL and Dollery CT (1976) Central and peripheral catecholamine mechanisms in circulatory control. Cardiology 61 suppl 1: 113-124

Richardson PJ, Kase H, and Jenner PG (1997) Adenosine A2A receptor antagonists as new agents for the treatment of Parkinson's disease. Trends Pharmacol Sci 18: 338-344

Rieger D, Auerbach S, Robinson P and Gropman A (2013) Neuroimaging of lipid storage disorders. Dev Disabil Res Rev. 2013 Jun;17(3):269-82

Riess O, Jakes R, and Kruger R (1998) Genetic dissection of familial Parkinson's disease. Mol Med Today 4: 438-444

Rinne UK, Bracco F, Chouza C, Dupont E, Gershanik O, Marti Masso JF, Montastruc JL, and Marsden CD (1998) Early treatment of Parkinson's disease with cabergoline delays the onset of motor complications. Results

of a double-blind levodopa controlled trial. The PKDS009 Study Group. Drugs 55 Suppl 1: 23-30

Rudzinska M and Szczudlik A (2007) [Apomorphine in off state--clinical experience]. Neurol Neurochir Pol 41: S40-S48

Samadi P, Rouillard C, Bedard PJ, and Di PT (2007) Functional neurochemistry of the basal ganglia. Handb Clin Neurol 83: 19-66

Sanghera MK, Grossman RG, Kalhorn CG, Hamilton WJ, Ondo WG, Jankovic J (2003) Basal ganglia neuronal discharge in primary and secondary dystonia in patients undergoing pallidotomy. Neurosurgery 52(6), 1358–1370; discussion 1370–1373

Schapira AH (2005) Present and future drug treatment for Parkinson's disease. J Neurol Neurosurg Psychiatry 76: 1472-1478

Schapira AH, McDermott MP, Barone P, Comella CL, Albrecht S, Hsu HH, Massey DH, Mizuno Y, Poewe W, Rascol O, Marek K (2013) Pramipexole in patients with early Parkinson's disease (PROUD): a randomised delayed-start trial. Lancet Neurol. 2013 Aug;12(8):747-55

Schneider JS, Yuwiler A, and Markham CH (1987) Selective loss of subpopulations of ventral mesencephalic dopaminergic neurons in the monkey following exposure to MPTP. Brain Res 411: 144-150

Schwartz DE, Jordan JC, and Ziegler WH (1974) Pharmacokinetics of the decarboxylase inhibitor benserazide in man; its tissue distribution in the rat. Eur J Clin Pharmacol 7: 39-45

Shannon KM, Goetz CG, Carroll VS, Tanner CM, and Klawans HL (1987) Amantadine and motor fluctuations in chronic Parkinson's disease. Clin Neuropharmacol 10: 522-526

Shen H, Kannari K, Yamato H, Arai A, and Matsunaga M (2003) Effects of benserazide on L-DOPA-derived extracellular dopamine levels and aromatic L-amino acid decarboxylase activity in the striatum of 6-hydroxydopamine-lesioned rats. Tohoku J Exp Med 199: 149-159

Shimura H, Hattori N, Kubo S, Mizuno Y, Asakawa S, Minoshima S, Shimizu N, Iwai K, Chiba T, Tanaka K, and Suzuki T (2000) Familial Parkinson disease gene product, parkin, is a ubiquitin-protein ligase. Nat Genet 25: 302-305

Shimazu S, Takahata K, Tamashiro A, Yoneda F, Iida Y, Saji H (2003) Recovery of motor function and dopaminergic parameters in a mouse model of Parkinson's disease induced by co-administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and diethyldithiocarbamate. J Neural Transm. 2003 Aug;110(8):871-83

Shindo H, Komai T, and Kawai K (1973a) Studies on the metabolism of D- and L-isomers of 3,4-dihydroxyphenylalanine (DOPA). V. Mechanism of

intestinal absorption of D- and L-DOPA-14C in rats. Chem Pharm Bull (Tokyo) 21: 2031-2038

Shindo H, Nakajima E, Kawai K, Miyakoshi N, and Tanaka K (1973b) Studies on the metabolism of D- and L-isomers of 3,4-dihydroxyphenylalanine (DOPA). 3. Absorption, distribution and excretion of D- and L-DOPA-14C in rats following intravenous and oral administration. Chem Pharm Bull (Tokyo) 21: 817-825

Singleton AB, Farrer MJ, Bonifati V (2013) The genetics of Parkinson's disease: progress and therapeutic implications. Mov Disord. 2013 Jan;28(1):14-23.

Smith LA, Jackson MJ, Hansard MJ, Maratos E, and Jenner P (2003) Effect of pulsatile administration of levodopa on dyskinesia induction in drug-naive MPTP-treated common marmosets: effect of dose, frequency of administration, and brain exposure. Mov Disord 18: 487-495

Smith LA, Jackson MJ, Johnston L, Kuoppamaki M, Rose S, Al-Barghouthy G, Del SS, and Jenner P (2006) Switching from levodopa to the long-acting dopamine D2/D3 agonist piribedil reduces the expression of dyskinesia while maintaining effective motor activity in MPTP-treated primates. Clin Neuropharmacol 29: 112-125

Sozio P, Cerasa LS, Abbadessa A, and Di SA (2012) Designing prodrugs for the treatment of Parkinson's disease. Expert Opin Drug Discov 7: 385-406

Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, and Goedert M (1997) Alpha-synuclein in Lewy bodies. Nature 388: 839-840

Spinnewyn B, Charnet C, Cornet S, Roubert V, Chabrier PE, Auguet M (2011) An improved model to investigate the efficacy of antidyskinetic agents in hemiparkinsonian rats. Fundam Clin Pharmacol. 2011 Oct;25(5):608-18

Stefanova N, Lundblad M, Tison F, Poewe W, Cenci MA, and Wenning GK (2004) Effects of pulsatile L-DOPA treatment in the double lesion rat model of striatonigral degeneration (multiple system atrophy). Neurobiol Dis 15: 630-639

Stocchi F (2003) Prevention and treatment of motor fluctuations. Parkinsonism Relat Disord 9 Suppl 2: S73-S81

Stocchi F, Tagliati M, and Olanow CW (2008) Treatment of levodopa-induced motor complications. Mov Disord 23 Suppl 3: S599-S612

Stocchi F, Vacca L, De Pandis MF, Barbato L, Valente M, and Ruggieri S (2001) Subcutaneous continuous apomorphine infusion in fluctuating patients with Parkinson's disease: long-term results. Neurol Sci 22: 93-94

Stocchi F, Vacca L, Grassini P, Battaglia G, Onofrj M, Valente M, and Ruggieri S (2003) Optimizing levodopa pharmacokinetics in Parkinson's disease: the role of COMT inhibitor. Neurol Sci 24: 217-218

Stockwell KA, Virley DJ, Perren M, Iravani MM, Jackson MJ, Rose S, and Jenner P (2008) Continuous delivery of ropinirole reverses motor deficits

without dyskinesia induction in MPTP-treated common marmosets. Exp Neurol 211: 172-179

Sundstrom E, Goldstein M, and Jonsson G (1986) Uptake inhibition protects nigro-striatal dopamine neurons from the neurotoxicity of 1-methyl-4-phenylpyridine (MPP+) in mice. Eur J Pharmacol 131: 289-292

Sundstrom E, Stromberg I, Tsutsumi T, Olson L, and Jonsson G (1987) Studies on the effect of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) on central catecholamine neurons in C57BL/6 mice. Comparison with three other strains of mice. Brain Res 405: 26-38

Surmeier DJ, Ding J, Day M, Wang Z, Shen W (2007) D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons. Trends Neuroscience, May;30(5):228-35

Tayarani-Binazir KA, Jackson MJ, Fisher R, Zoubiane G, Rose S and Jenner P (2010) The timing of administration, dose dependence and efficacy of dopa decarboxylase inhibitors on the reversal of motor disability produced by L-DOPA in the MPTP-treated common marmoset. Eur J Pharmacol. 2010 Jun 10;635(1-3):109-16

Terrón MP, Delgado-Adámez J, Pariente JA, Barriga C, Paredes SD, Rodríguez AB (2013) Melatonin reduces body weight gain and increases nocturnal activity in male Wistar rats. Physiol Behav. 2013 Jun 13;118:8-13

Tobón-Velasco JC, Silva-Adaya D, Carmona-Aparicio L, García E, Galván-Arzate S, Santamaría A (2010) Early toxic effect of 6-hydroxydopamine on extracellular concentrations of neurotransmitters in the rat striatum: an in vivo microdialysis study. Neurotoxicology. 2010 Dec;31(6):715-23

Thanvi B1, Lo N, Robinson T (2007) Levodopa-induced dyskinesia in Parkinson's disease: clinical features, pathogenesis, prevention and treatment. Postgrad Med J. 2007 Jun;83(980):384-8.

Thomas A, Iacono D, Luciano AL, Armellino K, Di IA, and Onofrj M (2004) Duration of amantadine benefit on dyskinesia of severe Parkinson's disease. J Neurol Neurosurg Psychiatry 75: 141-143

Tintner R, Manian P, Gauthier P, and Jankovic J (2005) Pleuropulmonary fibrosis after long-term treatment with the dopamine agonist pergolide for Parkinson Disease. Arch Neurol 62: 1290-1295

Torchiana ML, Lotti VJ, Clark CM, and Stone CA (1973) Comparison of centrally mediated hypotensive action of methyldopa and DOPA in cats. Arch Int Pharmacodyn Ther 205: 103-113

Treseder SA, Jackson M, and Jenner P (2000) The effects of central aromatic amino acid DOPA decarboxylase inhibition on the motor actions of L-DOPA and dopamine agonists in MPTP-treated primates. Br J Pharmacol 129: 1355-1364

Treseder SA, Rose S, Summo L, Jenner P (2003) Commonly used L-amino acid decarboxylase inhibitors block monoamine oxidase activity in the rat. J Neural Transm. 2003 Mar;110(3):229-38.

Tye SJ, Rupniak NM, Naruse T, Miyaji M, and Iversen SD (1989) NB-355: a novel prodrug for L-DOPA with reduced risk for peak-dose dyskinesias in MPTP-treated squirrel monkeys. Clin Neuropharmacol 12: 393-403

Ungerstedt U (1968) 6-Hydroxy-dopamine induced degeneration of central monoamine neurons. Eur J Pharmacol 5: 107-110

Ungerstedt U and Arbuthnott GW (1970) Quantitative recording of rotational behavior in rats after 6-hydroxy-dopamine lesions of the nigrostriatal dopamine system. Brain Res 24: 485-493

Ursan ID, Chiu L, Pierce A (2003) Three-dimensional dru g printing: a structured review. J Am Pharm Assoc (2003). 2013 Mar-Apr;53(2):136-44

Valente EM, bou-Sleiman PM, Caputo V, Muqit MM, Harvey K, Gispert S, Ali Z, Del TD, Bentivoglio AR, Healy DG, Albanese A, Nussbaum R, Gonzalez-Maldonado R, Deller T, Salvi S, Cortelli P, Gilks WP, Latchman DS, Harvey RJ, Dallapiccola B, Auburger G, and Wood NW (2004a) Hereditary early-onset Parkinson's disease caused by mutations in PINK1. Science 304: 1158-1160

Valente EM, Salvi S, Ialongo T, Marongiu R, Elia AE, Caputo V, Romito L, Albanese A, Dallapiccola B, and Bentivoglio AR (2004b) PINK1 mutations

are associated with sporadic early-onset parkinsonism. Ann Neurol 56: 336-341

Van CG, Flamez A, Cosyns B, Weytjens C, Muyldermans L, Van ZM, De SJ, Santens P, Decoodt P, Moerman C, and Schoors D (2004) Treatment of Parkinson's disease with pergolide and relation to restrictive valvular heart disease. Lancet 363: 1179-1183

Verhagen ML, Del DP, van den MP, Fang J, Mouradian MM, and Chase TN (1998) Amantadine as treatment for dyskinesias and motor fluctuations in Parkinson's disease. Neurology 50: 1323-1326

Vickers S, Stuart EK, Bianchine JR, Hucker HB, Jaffe ME, Rhodes RE, and Vandenheuvel WJ (1974) Metabolism of carbidopa (1-(-)-alpha-hydrazino-3,4-dihydroxy-alpha-methylhydrocinnamic acid monohydrate), an aromatic amino acid decarboxylase inhibitor, in the rat, rhesus monkey, and man. Drug Metab Dispos 2: 9-22

van den Munckhof P, Luk KC, Ste-Marie L, Montgomery J, Blanchet PJ, Sadikot AF and Drouin J (2003) Pitx3 is required for motor activity and for survival of a subset of midbrain dopaminergic neurons. Development. 2003 Jun;130(11):2535-42

VINCENT WA, KASHEMSANT U, CUDDY RP, SMULYAN H and EICH RH (1963) THE ACUTE HEMODYNAMIC EFFECTS OF L-ALPHA METHYLDOPA. Am J Med Sci. 1963 Nov;246:558-68

Voon V, Dalley JW (2011) Parkinson disease: impulsive choice-Parkinson disease and dopaminergic therapy. Nat Rev Neurol. 2011 Sep 13;7(10):541-2

Waters C (2013) The development of the rotigotine transdermal patch: a historical perspective. Neurol Clin. 2013 Aug;31(3 Suppl):S37-50

Watts RL (1997) The role of dopamine agonists in early Parkinson's disease. Neurology 49: S34-S48

WEINER N, PERKINS M, and SIDMAN RL (1962) Effect of reserpine on noradrenaline content of innervated and denervated brown adipose tissue of the rat. Nature 193: 137-138

Willis AW Parkinson disease in the elderly adult (2013) Mo Med. Sep Oct;110(5):406-10. Review

Wood LD (2010) Clinical review and treatment of select adverse effects of dopamine receptor agonists in Parkinson's disease. Drugs Aging 27: 295-310

Wood LD, Neumiller JJ, Setter SM, and Dobbins EK (2010) Clinical review of treatment options for select nonmotor symptoms of Parkinson's disease. Am J Geriatr Pharmacother 8: 294-315

Wooten GF (2001) Anatomy and function of dopamine receptors: understanding the pathophysiology of fluctuations in Parkinson's disease. Parkinsonism Relat Disord 8: 79-83

Wooten GF (2003) Agonists vs levodopa in Parkinson's disease: the thrilla of whitha. Neurology 60: 360-362

Wurtman RJ and Ordonez LA (1978) Effects of exogenous L-dopa on the metabolism of methionine and S-adenosylmethionine in the brain. Monogr Gesamtgeb Psychiatr Psychiatry Ser 18: 132-143

Yacoubian TA (2013) IPX066: a new intermediate-and extended-release carbidopa-levodopa formulation. Neurodegener Dis Manag. 2013 Apr 1;3(2):123-131

Yitey-Smith E and Varma DR (1970a) An assessment of the role of the sympathetic nervous system in experimental hypertension using normal and immunosympathectomized rats. Br J Pharmacol 40: 175-185

Yitey-Smith E and Varma DR (1970b) Mechanism of the hypotensive action of methyldopa in normal and immunosympathectomized rats. Br J Pharmacol 40: 186-193

Zádori D, Szalárdy L, Toldi J, Fülöp F, Klivényi P, Vécsei L (2013) Some molecular mechanisms of dopaminergic and glutamatergic dysfunctioning in Parkinson's disease. J Neural Transm. 2013 Apr;120(4):673-81

Zaleska B and Domzal T (1999) [Apomorphine in treatment of Parkinson's disease with fluctuations]. Neurol Neurochir Pol 33: 1297-1303

Zhou T, Hider RC, Jenner P, Campbell B, Hobbs CJ, Rose S, Jairaj M, Tayarani-Binazir KA, and Syme A (2010) Design, synthesis and biological evaluation of L-dopa amide derivatives as potential prodrugs for the treatment of Parkinson's disease. Eur J Med Chem 45: 4035-4042

Ziegler M and Szechtman H (1988) Differences in the behavioral profile of circling under amphetamine and apomorphine in rats with unilateral lesions of the substantia nigra. Behav Neurosci 102: 276-88, 327

Zubair M, Jackson MJ, Tayarani-Binazir K, Stockwell KA, Smith LA, Rose S, Olanow W, and Jenner P (2007) The administration of entacapone prevents L-dopa-induced dyskinesia when added to dopamine agonist therapy in MPTP-treated primates. Exp Neurol 208: 177-184